

**Motor learning and neuroplasticity
in humans**

by

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**A thesis submitted to UCL for
a PhD in the Faculty of Science.**

June 2009.

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**Word Count: 56103 (chapters only)
58621 (preface and chapters only)**

Declaration

I, James TEO Teong Han, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

The central nervous system is plastic, in that the number and strength of synaptic connections changes over time. In the adult the most important driver of such changes is experience, in the form of learning and memory. There are thought to be a number of rules, operating relatively local to each synapse that govern changes in strength and organisation. Some of these such as Hebbian plasticity or plasticity following repeated activation of a connection have been studied in detail in animal preparations. However, recent work with non-invasive methods of transcranial stimulation in human, such as transcranial magnetic stimulation, has opened the opportunity to study similar effects in the conscious human brain.

In this thesis I use these methods to explore some of the presumed changes in synaptic connectivity in the motor cortex during different forms of motor learning. The experiments only concern learning in the healthy brain; however it seems likely that the same processes will be relevant to neurorehabilitation and disease of the nervous system.

This thesis explores the link between neuroplasticity and motor learning in humans using non-invasive brain stimulation, pharmacological agents and psychomotor testing in 6 related studies.

- 1) Chapter 3 reports initial pharmacological investigations to confirm the idea that some of the long term effects of TMS are likely to involve LTP-like mechanisms. The study shows that NMDA agonism can affect the response to a repetitive form of TMS known as theta burst stimulation (TBS)
- 2) Following up on the initial evidence for the role of NMDA receptors in the long term effects of TBS, Chapter 4 explores the possible modulatory effects of dopaminergic drugs on TBS.
- 3) Chapter 5 takes the investigations to normal behaviours by examining how the NMDA dependent plasticity produced by TBS interacts with learning a simple motor task of rapid thumb abduction. The unexpected results force a careful examination of the possible mechanisms of motor learning in this task.
- 4) Chapter 6 expands on these effects by employing a battery of TMS methods as well as drug agents to examine the role of different intracortical circuits in ballistic motor learning.
- 5) Chapter 7 studies the plasticity of intracortical circuits involved in transcallosal inhibition.
- 6) Chapter 8 studies the interaction between synaptic plasticity invoked by TBS and sequence learning.

The studies described in the thesis contribute to understanding of how motor learning and neuroplasticity interact, and possible strategies to enhance these phenomena for clinical application.

Acknowledgements

This thesis is dedicated to my wife, Ping – who kept me focused and writing this thesis even when I had lost the motivation. She has also supported my lunatic interests for which I am grateful for.

It is also not possible to thank enough the sacrifices that my parents have made to ensure that I was educated enough to be able to write this thesis.

And my supervisors, John Rothwell and Richard Greenwood, who nudged me every so often in the right direction.

Contents

Abstract	iii
Acknowledgement	iv
Contents	v
Figures and Tables	xiii
Abbreviations	xvi
Publications in relation to this thesis	xviii
 Chapter 1 – Introduction	 1
1.1 Plasticity	4
1.1.1 Long term potentiation (LTP) / Hebbian plasticity	5
1.1.1.1 Hebbian features of LTP/ LTD	7
1.1.1.2 Non-classical LTP	7
1.1.2 Structural plasticity	8
1.1.3 Metaplasticity	9
1.1.4 Homeostatic plasticity	11
1.1.5 Brain-derived neurotrophic factor (BDNF) and neuroplasticity	12
1.2 The study of plasticity in humans	12
1.2.1 Transcranial magnetic stimulation	13
1.2.2 Repetitive transcranial magnetic stimulation	16
1.2.2.1 Theta burst stimulation	16
1.2.2.2 I-wave interval rTMS	18
1.2.3 Paired associative stimulation	18
1.2.4 Transcranial direct current stimulation	19

1.2.5	Direct cortical stimulation	20
1.2.6	Similarities between human and animal neuroplasticity models	20
1.3	Drugs in the study of plasticity	22
1.3.1	Noradrenergic drugs	22
1.3.2	Dopaminergic drugs	23
1.3.3	Cholinergic drugs	24
1.3.4	GABA-ergic drugs	25
1.3.5	Endocannabinoids	26
1.3.6	Glutamergic drugs	27
1.4	Motor learning	28
1.4.1	Motor learning paradigms	29
1.4.1.1	Sequence learning	30
1.4.1.2	Ballistic motor learning	30
1.4.1.3	Visuomotor transformations	31
1.4.1.4	Force field adaptation	32
1.4.1.5	Locomotor adaptation	33
1.4.1.6	Classical conditioning	33
1.4.1.7	Aimed rapid movements	34
1.4.2	Explicit learning in motor learning	36
1.4.3	Adaptation versus skill-learning	36
1.4.4	Stages of motor learning	37
1.4.5	Summary of motor learning	39
1.5	Relationship between plasticity and motor learning	39
1.5.1	Evidence from animal models	39

1.5.2	Evidence from humans	41
1.6	Motor learning and plasticity in disease	43
1.6.1	Stroke	43
1.6.1.1	Stroke recovery through neuroplasticity and motor learning	44
1.6.1.2	The ‘hemispheric rivalry’ hypothesis	46
1.6.2	Parkinson’s Disease	47
1.6.2.1	Levodopa-associated dyskinesias	49
1.6.3	Cerebellar disease	49
1.6.4	Dystonia	50
1.6.5	Huntington’s disease	51
1.6.6	Alzheimer’s disease	52
1.6.7	Relevance of motor learning to neurorehabilitation	53
1.7	Goal of this thesis	54
Chapter 2 – General Methods		56
2.1	Subjects	57
2.2	Electromyography	57
2.3	Transcranial magnetic stimulation	58
2.4	Repetitive transcranial magnetic stimulation	59
2.5	Behavioural measures	60
2.6	EMG data analysis	60
2.7	Statistical analysis	61
Chapter 3 – NMDA agonism and theta burst stimulation		62

3.1	Introduction	63
3.2	Study design	64
3.3	Drug	65
3.4	Results	67
3.5	Discussion	68
Chapter 4 – Theta burst stimulation and neuromodulatory drugs		71
4.1	Introduction	72
4.2	Amphetamine & levo-dopa	72
4.2.1	Study Design	73
4.2.1.1	Drugs	74
4.2.1.2	Statistical analysis	74
4.2.2	Results	74
4.2.3	Discussion	78
4.3	Nicotine	79
4.3.1	Study Design	80
4.3.1.1	Drug	80
4.3.1.2	Statistical analysis	81
4.3.2	Results	81
4.3.3	Discussion	85
Chapter 5 – Theta burst stimulation and ballistic motor learning		86
5.1	Introduction	87
5.2	Study design	87

5.2.1	Ballistic motor learning	88
5.2.2	Data analysis	89
5.3	Results	90
5.4	Discussion in the interlude	95
5.4.1	The effect of iTBS on motor learning	95
5.4.2	The effect of the iTBS-nicotine interaction on learning	96
5.4.3	Trial-by-trial analysis of data	97
5.5	Modelling the ballistic motor learning task	99
5.7.1	Implications of the model	105
5.6	Analysing variability of performance during learning	107
5.7	Control experiment: TBS on variability of TMS-evoked movements	111
5.8	Conclusion	114
Chapter 6 – Intracortical circuits and practice-dependent plasticity		117
6.1	Introduction	118
6.2	Study design	119
6.3	TMS measurements	121
6.4	Motor practice	123
6.5	Results	124
6.5.1	Drug-induced changes	125
6.5.2	Practice	128
6.5.3	Practice-induced changes	129
6.5.4	Correlations	132
6.6	Discussion	134
6.6.1	Drug induced changes in cortical circuits	134

6.6.2	Practice-dependent plasticity	135
6.6.3	Link between SAI and perception variability	138
6.6.4	Conclusion	139
Chapter 7 – Intracortical circuits and transcallosal pathways		140
7.1	Introduction	141
7.2	Intracortical circuits that modulate transcallosal inhibition	142
7.2.1	Study design	142
7.2.2	Data analysis	145
7.2.2.1	Data analysis of iSP	145
7.2.2.2	Data analyses of IHI	146
7.2.2.3	Data analyses of cMEP	146
7.2.2.4	Statistical analysis	147
7.2.3	Results	147
7.2.3.1	Demonstration of SICF-like effects on iSP	147
7.2.3.2	Demonstration of SICF-like effects on IHI	155
7.2.4	Discussion	160
7.2.4.1	Site of facilitatory interaction	161
7.2.4.2	Nature of facilitatory interaction	159
7.3	Effect of rTMS on transcallosal circuits	163
7.3.1	Study design	164
7.3.2	Data analysis	165
7.3.3	Results	166
7.3.3.1	SICI _{cMEP} and SICI _{iSP}	167
7.3.3.2	Effect of rTMS on SICI _{cMEP} and SICI _{iSP}	170

7.3.4	Discussion	172
7.4	Conclusion	174
Chapter 8 – Theta burst stimulation and sequence learning		175
8.1	Introduction	176
8.2	Methods	179
8.2.1	Participants	179
8.2.2	Serial Reaction Time (SRT) Task	180
8.2.3	Theta burst stimulation	182
8.2.4	SRT task data analysis	184
8.3	Results	184
8.3.1	Reaction Times	185
8.3.2	Variability of Reaction Times	191
8.3.3	Errors	191
8.4	Discussion	192
8.4.1	Methodological differences across studies of the effects of rTMS and tDCS on SRT learning	192
8.4.2	Neural basis of motor sequence learning	194
8.4.3	The role of the M1 and the SMA in sequential learning.	195
8.4.4	Why doesn't excitatory TBS produce enhanced learning?	196
8.4.5	The lack of reaction time improvement in probabilistic sequence learning	197
8.5	Conclusion	198
Chapter 9 – Conclusion		199
9.1	Summary	200

9.2	Brain stimulation, motor learning and plasticity	201
9.3	Future possible studies	202
9.3.1	Studying the relationship between variability and plasticity	202
9.3.2	Studying the role of motor learning in neurorehabilitation	203
9.3.3	Studying the role of endocannabinoids in human neuroplasticity	203
9.4	Closing statements	204
Appendix: References		206

Figure and Tables

Figure/ Table	Description	Page
Figure 1.1	Frequency and calcium-dependency of classical LTP/ LTD	6
Figure 1.2	Graphical representation of the Bienenstock-Cooper-Munro learning rule	10
Figure 1.3	Example of epidural volleys and motor-evoked potentials from a single TMS pulse	14
Table 1.1	Summary of various paired-pulse TMS measurements	15
Figure 1.4	Pictorial representation of cTBS and iTBS repetitive TMS	17
Figure 3.1	The effect of d-cycloserine or placebo on iTBS repetitive TMS	66
Table 3.1	Baseline measures for d-cycloserine or placebo experimental arms	67
Table 4.1	Baseline measures for amphetamine, levodopa or placebo experimental arms	75
Figure 4.1	The effect of amphetamine or placebo on iTBS repetitive TMS	76
Figure 4.2	The effect of levodopa or placebo on iTBS repetitive TMS	77
Table 4.2	Baseline measures for nicotine or placebo experimental arms	81
Figure 4.3	The effect of nicotine or placebo on iTBS repetitive TMS	83
Figure 4.4	The effect of nicotine on motor-evoked potentials	84
Figure 5.1	The effect of nicotine and iTBS on repeated peak initial acceleration of left thumb abduction	91
Table 5.1	Analysis of variance (ANOVA) of ballistic motor learning	93-94
Table 5.2	Analysis of variance (ANOVA) of baseline ballistic performance	94
Figure 5.2	Probability of an individual trial being better than previous trial	98
Figure 5.3	Model of a possible strategy for the ballistic learning task	101-102
Figure 5.4	The interaction of performance variability, perception variability and learning gain in the ballistic learning model	104-105
Figure 5.5	Example of trial-to-trial measure of performance variability	108

Figure 5.6	Performance variability during the learning task (group means of each session)	110-111
Figure 5.7	The effect of iTBS on the variability of TMS-evoked thumb movements	113-114
Figure 6.1	Study design of the role of GABA-circuits in practice-dependent plasticity	120
Table 6.1	Subject and TMS parameters at T1 (before drug)	124
Table 6.2	Subject and TMS parameters at T2 (after drug)	125
Table 6.3	Subject and TMS parameters at T3 (after training)	125
Figure 6.2	The effect of drug on TMS measurements	126
Figure 6.3	Motor performance during training	129
Figure 6.4	The effect of training on TMS measurements	130-131
Figure 6.5	Correlation analysis of the drug-induced and practice-induced changes	133
Figure 7.1	Study design of transcallosal experiments 1a and 1b	142
Figure 7.2	Example of MEPs and ipsilateral silent period of two stimuli delivered at different ISI	148
Figure 7.3	Group means of ipsilateral silent period and contralateral MEP from single and paired TMS pulses	150-151
Table 7.1	Statistical analysis of experiment 1a	152
Figure 7.4	Correlations between iSP and cMEP	154
Figure 7.5	Group means of interhemispheric inhibition and contralateral MEP from single and paired TMS pulses	156
Table 7.2	Statistical analysis of experiment 1b	158
Figure 7.6	Correlations between IHI and cMEP	159
Figure 7.7	Study design of experiment 2 to study the effect of rTMS on the inhibitory intracortical circuits	164
Figure 7.8	Effect on conditioning stimulus on cMEP and iSP	168
Table 7.3	Statistical analysis of effect on conditioning stimulus on cMEP and iSP	169-170
Table 7.4	Baseline characteristics in experiment 2	170
Figure 7.9	Effect of rTMS on cMEP and iSP	171

Table 7.5	Statistical analysis of the effect of rTMS on cMEP and iSP	172
Table 7.6	Statistical analysis of the effect of rTMS on $SICI_{cMEP}$ with $SICI_{iSP}$	173
Figure 8.1	Mean RTs across training blocks	185-186
Figure 8.2	Mean RT difference scores by epoch	188-189

Abbreviations

CTS	Corticospinal
TC	Transcallosal
CS	Conditioning stimulus
TS	Test stimulus
iSP	Ispilateral silent period
IHI	Interhemispheric inhibition
SICF	Short-interval intracortical facilitation
SIC _{I_cMEP}	Short-interval intracortical inhibition of the corticospinal pathway
SIC _{I_iSP}	Short-interval intracortical inhibition of the transcallosal pathway
SIC _{I_{2ms}}	Short-interval intracortical inhibition with 2ms interstimulus interval
SIC _{I_{3ms}}	Short-interval intracortical inhibition with 3ms interstimulus interval
SIC _{I_{comb}}	Short-interval intracortical inhibition averaged for 2ms and 3ms interstimulus intervals
ICF	Intracortical facilitation
SAI	Short latency afferent inhibition
LAI	Long latency afferent inhibition
cMEP	Contralateral motor-evoked potential
MEP	Motor-evoked potential
RMT	Resting motor threshold
AMT	Active motor threshold
MSO	Maximum stimulator output
rTMS	Repetitive transcranial magnetic stimulation
TMS	Transcranial magnetic stimulation
TBS	Theta-burst stimulation
iTBS	Intermittent theta burst stimulation
cTBS	Continuous theta burst stimulation
FDI	First dorsal interosseus
APB	Abductor pollicis brevis
ADM	Adductor digiti minimi

GABA	Gamma-aminobutyric acid
AChR	Acetylcholine receptor
PAS	Paired associative stimulation
TDCS	Transcranial direct current stimulation
ISI	Interstimulus interval
IQ	Intelligence quotient
SMA	Supplementary motor area
DLPFC	Dorsolateral prefrontal cortex
M1	Primary motor cortex
SRT	Serial reaction time
PD	Parkinson's Disease
RT	Reaction time
EMG	Electromyography

Publications in relation to this thesis

The following publications have come from work recorded in this thesis:

- Teo JT, Swayne OB, Rothwell JC. Further evidence for NMDA-dependence of the after-effects of human theta burst stimulation. Clin Neurophysiol. 2007 Jul; 118(7):1649-51.
I am the lead author of this paper as I contributed substantially in the design, concept and interpretation of the study. Data from this paper is used in Chapter 3.
- Teo JT, Terranova C, Swayne OB, Greenwood R, Rothwell JC. Practice-dependent plasticity is limited by different intracortical circuits. Exp Brain Res. 2009 Mar;193(4):555-63.
I am the lead author of this paper as I contributed substantially in the design, concept and interpretation of the study. Data from this paper is used in Chapter 6.
- Avanzino L, Teo JT, Rothwell JC. Intracortical circuits modulate transcallosal inhibition in humans. J Physiol. 2007 Aug 15; 583(Pt 1):99-114.
I am one of the shared lead authors of this paper as I contributed substantially in the design, concept and interpretation of the studies. Data from this paper is used in Chapter 7.
- Wilkinson L, Teo JT, Obeso I, Rothwell JC, Jahanshahi M. The contribution of the primary motor cortex is essential for probabilistic implicit sequence learning: evidence from theta burst magnetic stimulation. J Cogn Neurosci. 2009 Mar 20. [Epub ahead of print]
I am the second author of this paper although I contributed substantially in the design, concept and interpretation of the study; especially the use of transcranial magnetic stimulation. Data from this paper is used in Chapter 8.

Accepted

- Swayne OB, Teo JT, Greenwood R, Rothwell JC. Nicotine modulates the effects of theta burst stimulation (accepted by Clinical Neurophysiology; 22nd June 2009).
I am the second author of this paper although I contributed substantially in the conduct of experiments and interpretation of the study. Data from this paper is used in Chapter 4.

Submitted

- Teo JT, Swayne OB, Cheeran BJ, Greenwood R, Rothwell JC. Human theta burst stimulation enhances subsequent motor learning while increasing performance variability (submitted to Cerebral Cortex; 27th June 2009).
I am the lead author of this paper and I contributed substantially in the design, concept, data collection, modeling design and interpretation of the study. Data from this paper is used in Chapter 5.

Chapter 1

Introduction

The central nervous system has a wide array of functions: receiving sensory input, storing memories, coordinating motor plans, maintaining posture, and generating consciousness and higher thought. The nervous system accomplishes this diversity of functions with one key feature: it can change and adapt. In this way, characteristics can be tuned to the task at hand and new properties can be acquired. This ability of the nervous system to change is perplexing as the adult nervous system generates relatively few new cells.

This dilemma was recognised by the great Spanish histologist and neuroscientist, Santiago Ramón y Cajal (1852-1934) who posited an explanation in his 1894 Croonian Lecture to the Royal Society in London:

"La gymnastique cérébrale n'est pas susceptible d'améliorer l'organisation du cerveau en augmentant le nombre de cellules, car, on le sait, les éléments nerveux ont perdu depuis l'époque embryonnaire la propriété de proliférer; mais on peut admettre comme une chose très vraisemblable que l'exercice mental suscite dans les régions cérébrales plus sollicitées un plus grand développement de l'appareil protoplasmique et du système des collatérales nerveuses. De la sorte, des associations déjà créées entre certains groupes de cellules se renforceraient notablement au moyen de la multiplication des ramilles terminales des appendices protoplasmiques et des collatérales nerveuses; mais, en outre, des connexions intercellulaires tout à fait nouvelles pourraient s'établir grâce à la néoformation de collatérales et d'expansions protoplasmiques."

- Cajal, The Croonian Lecture (1894)

“Cerebral acrobatics cannot improve the organisation of the brain by increasing the number of cells because, since their embryological stages, the elements of the nervous system have lost the ability to multiply themselves; but it seems very likely that mental exercise engenders greater expansion of the dendritic apparatus and the system of axonal collaterals. In this way, connections already established between certain groups of cells would be particularly strengthened by the multiplication of the small terminal branches of the dendritic appendages and axonal collaterals; moreover, new intercellular connections could be established thanks to the formation of new collaterals and dendrites”

- English translation

However, Cajal’s prescient proposal – that it is not new cells which are generated but new pathways and synapses – was overshadowed by his view 20 years later that:

“nerve paths are something fixed, ended, immutable.

Everything may die, nothing may be regenerated”

- Cajal (1913-1914)

And this view of a rigid, static nervous system prevailed for most of the 20th century. In the later half of the 20th century, more evidence began to mount to demonstrate that the central nervous system does indeed adapt and is mutable even in adulthood; this broad idea is commonly termed neuroplasticity.

1.1 Plasticity

Neuroplasticity using the broadest definition is the ability of neurons (or the nervous system) to rearrange their anatomical and functional connectivity and properties in response to environmental input. This broad definition encompasses functional, structural, physiological and molecular changes but the most interesting form of neuroplasticity is neuroplasticity that obeys Hebbian rules as first described by Daniel Hebb:

When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.

- Hebb (1949)

This description has often been simplified to the more pithy “neurons that fire together, wire together”. Hebbian principles form the mathematical basis of neural network models and provide a principle that governs neuroplasticity, allowing synapses to retain a memory of previous activity.

The first clues for the molecular basis of how a nervous system can display neuroplasticity and adapt its motor behaviour was found in the invertebrate sea-slug, *Aplysia californica* by Eric Kandel and his group in 1969-1973 (Frazier et al., 1969; Kupfermann et al., 1970; Castelluci et al., 1970; Pinkser et al., 1973): changes in synaptic properties were shown to occur after the *Aplysia californica* had acquired a memory. This led to the discovery of long-term potentiation (LTP) in the mammalian hippocampus

1973 by Bliss & Lømo, which provided a molecular mechanism for neuroplasticity which obeys Hebbian principles.

1.1.1 Long term potentiation (LTP) / Hebbian plasticity

Long term potentiation (LTP) was first described as the long-lasting increase in synaptic efficacy after tetanic stimulation of the presynaptic neuron (review: Collingridge & Bliss, 1987; Collingridge & Bliss, 1995; Bliss et al., 2004). This long-lasting change is due to presynaptic neurotransmitter release and postsynaptic receptor expression and the key trigger is the NMDA glutamate receptor.

The NMDA receptor is a ligand-gated calcium channel. It has a binding site for glutamate on the extracellular surface which gates the opening of the channel. Additionally, the channel pore is also blocked by a Mg^{2+} ion, which has to be first displaced by depolarisation of the postsynaptic neuron, before the channel is fully open. In this way, the NMDA receptor acts as a 'coincidence detector' for presynaptic and postsynaptic depolarisation, and allows LTP to obey Hebbian principles (Bliss et al., 2004).

The transient rise in intracellular Ca^{2+} concentration serves to activate Ca^{2+} -dependent enzymes, Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC). These enzymes phosphorylate various proteins and receptors, and crucially the cAMP-response-element-binding-protein (CREB) which triggers CREB-dependent gene expression (Yin & Tully, 1996; Ahmed & Frey, 2004). Presynaptic processes also mediate LTP, and retrograde messengers such as nitric oxide and endocannabinoids deliver the message to the presynaptic cell to increase or decrease synaptic vesicle fusion (Arancio et al., 1996; Kanto et al., 1996; Wilson et al., 2001).

Long term depression (LTD) is the corollary of LTP with reduction of synaptic efficacy after lower frequency repetitive stimulation, and this is also dependent on the NMDA receptor (Dudek & Bear, 1992; Dudek & Bear, 1993). The determinant for whether LTP or LTD is induced is the intracellular Ca^{2+} concentration which binds differentially to C and N lobes of the calmodulin kinase depending on the rise of Ca^{2+} (Fig 1.1)

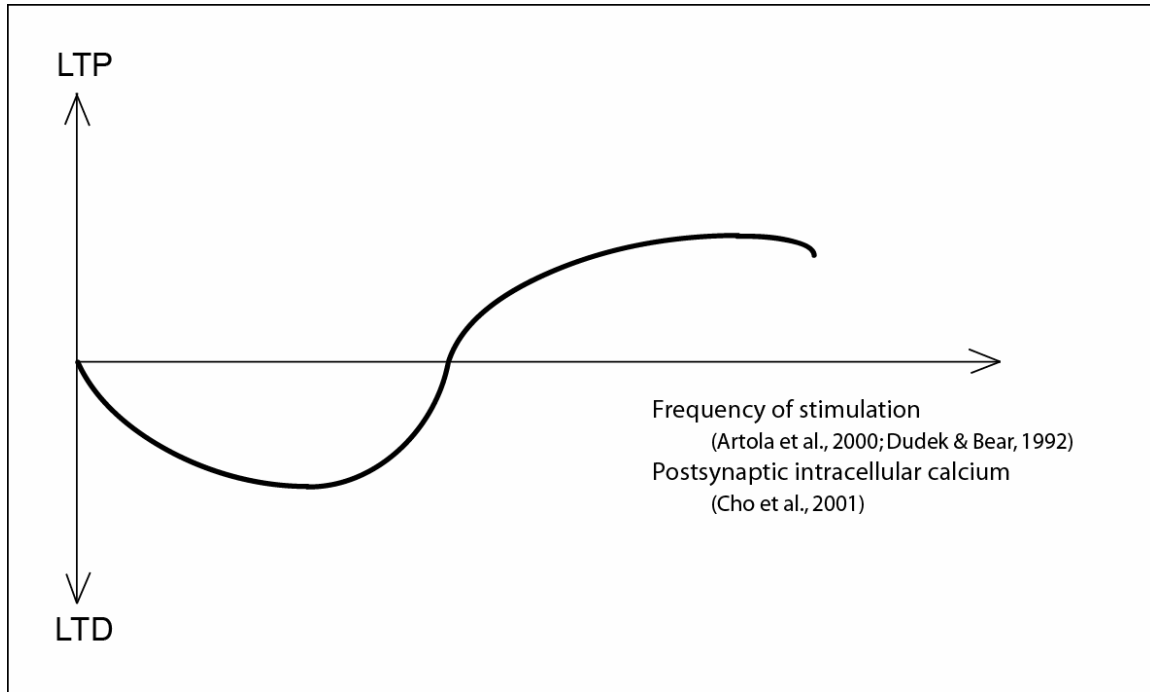


Fig 1.1 Frequency and calcium-dependency of classical LTP/ LTD where the ordinate represents change in synaptic efficacy, and abscissa can represent either frequency of induction stimulation or postsynaptic intracellular calcium.

Neuroplasticity operating on similar molecular pathways has since been discovered with other more physiologically-realistic forms of experimental stimulation: theta frequency stimulation (Larson et al., 1986; Larson et al., 1988) and spike-timing-dependent plasticity, STDP (Markram et al., 1997). It has also been found in different brain regions

including: striatum (Charpier & Deniau, 1997; Fino et al., 2005) and sensorimotor cortex (Hess & Donoghue, 1994; Hess et al., 1996), thus verifying its physiological relevance to the brain.

1.1.1.1 Hebbian features of LTP/ LTD

The key features of LTP/ LTD which make it an attractive molecular mechanism for Hebbian neuroplasticity are:

- (a) Rapid induction: LTP/ LTD can be induced rapidly by one or more brief tetanic stimuli;
- (b) Input specificity: LTP/ LTD once induced occurs only at inputs which have been stimulated;
- (c) Associativity: Weak inputs can produce LTP/ LTD in the presence of strong inputs depending on precise timing (spike-timing dependency);
- (d) Cooperativity: Multiple weak inputs can summate in space and/ or time (frequency-dependency) to produce LTP/ LTD; and
- (e) Long-lasting: The effects are immediate and last several hours.

These characteristics of LTP/ LTD govern neural network and computational models based on Hebbian principles, and also set a benchmark for assessing other models of neuroplasticity.

1.1.1.2 Non-classical LTP

There are many other forms of synaptic plasticity that do not conform to the above classical form of LTP / LTD. One such variant is the synaptic plasticity in the parallel

fibre and Purkinje cell synapse in the cerebellum (Ito et al., 2002; Jörntell & Hansel, 2006) as described by the Marr-Albus model (Marr, 1969; Albus, 1971). This model is beyond the scope of this thesis but broadly follows Hebbian rules at the synapse between the parallel fibre and Purkinje cells with LTD (instead of LTP) occurring when there is coincident activation of the parallel-fibre-Purkinje cell synapse and climbing fibre synapse (Ito et al., 1982; Ito et al., 2006).

Other variants of non-classical LTP also exist with LTP at inhibitory synapses in the developing visual cortex (Komatsu, 1996; Komatsu & Yoshimura, 2000) and non-NMDA-dependent LTP (Cavus & Teyler, 1998; Grover & Yan, 1999). It is worthwhile noting though that all these non-classical types of LTP identified still appear to broadly follow Hebbian rules.

1.1.2 Structural plasticity

The previous forms of neuroplasticity suggest a change in the properties of synapses between neurons. However, the past decade have provided evidence for the unmasking of silent synapses (Geinisman et al., 1996; Atwood & Wojtowicz, 1999; Ward et al., 2006; Itami et al., 2003) and new synapse formation (Geinisman et al., 1991) associated with LTP-induction indicating structural neuroplasticity after neuronal stimulation. Dendritic spines and synaptic boutons are extremely dynamic in animals, and changes have been shown to be associated with experience (Lendvai et al., 2000; Knott et al., 2002; Trachtenburg et al., 2002) and associative learning (Geinisman et al., 2001) in a number of brain regions. These changes in dendritic spine morphology and synaptogenesis have

also been shown to be linked with the induction of LTP (Toni et al., 1999; Engert et al., 1999), thereby providing a link to Hebbian principles discussed earlier.

Another form of structural plasticity is neurogenesis. While it has been accepted for most of the 20th century that no new neurons are formed in the adult brain, this has been shown to be false. It is now widely accepted that neurogenesis does occur in the adult hippocampus and the olfactory bulb (Altman & Das, 1967; Eriksson et al., 1998; Bernier et al., 2002). There is some limited evidence that neurogenesis also occurs in other brain regions (e.g. neocortex, striatum, amygdala) (Gould et al., 1999; Magavi et al., 2000; Dayer et al., 2005) although this remains controversial (Rakic et al., 1985; Bhardwarj et al., 2006). Also how widespread this phenomenon is and whether it participates in learning and memory remains controversial (Leuner et al., 2006; Gould et al., 2007; Cameron & Dayer 2008).

1.1.3 Metaplasticity

One consequence of the Hebbian characteristics of LTP / LTD is that it is inherently unstable. This was first predicted by Bienenstock, Cooper & Munro (1982) after modelling the development of orientation and binocular selectivity of neurons in the visual cortex, showing that synapses in a purely Hebbian model would segregate into maximally saturated synapses via LTP and maximally desaturated synapses via LTD. They proposed the BCM learning rule: recent high synaptic activity makes LTP harder to induce and LTD easier to induce and vice versa with recent low synaptic activity (Bienenstock et al., 1982; Wexler & Stanton, 1993). The mechanics of the BCM rule is summarised by Fig 1.2.

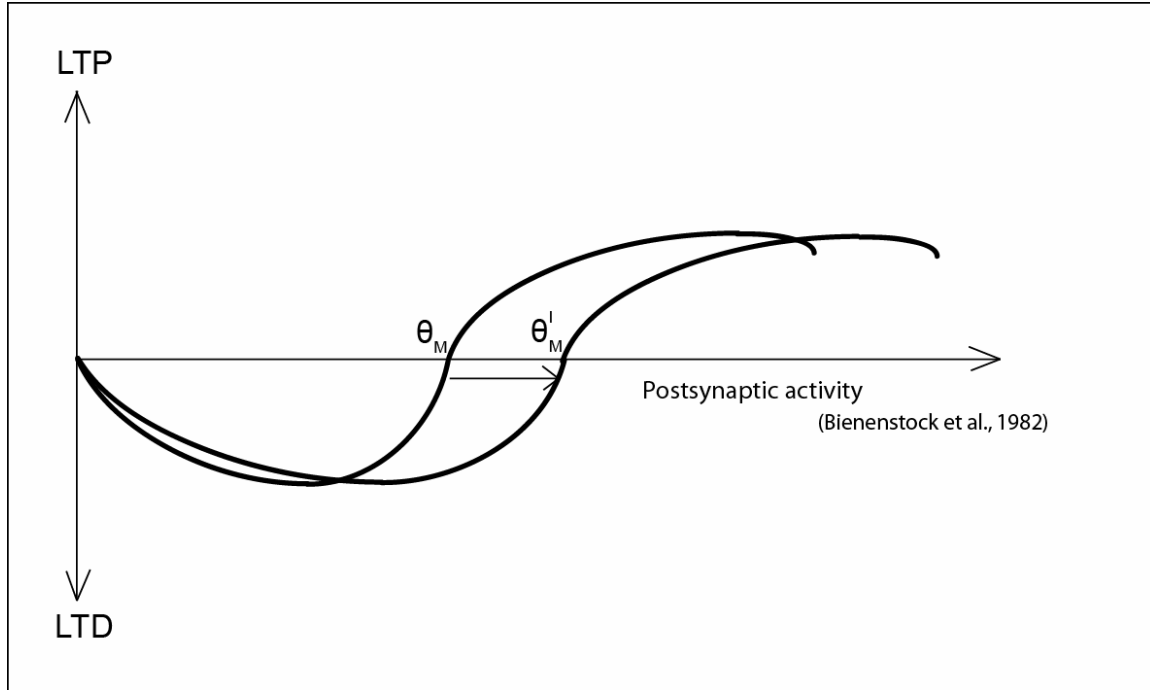


Fig 1.2 Graphical representation of the Bienenstock-Cooper-Munro learning rule where the ordinate represents change in synaptic efficacy, and abscissa can represent either postsynaptic activity, frequency of induction stimulation or postsynaptic intracellular calcium. The crossover point between inducing LTP or LTD is θ_M and slides as a function of previous synaptic activity.

The threshold for inducing LTP or LTD, θ_M , 'slides' horizontally and moves to the left after periods of low synaptic activity and moves to the right after periods of high synaptic activity. This places a negative feedback to changes of synaptic gain to prevent runaway LTP-processes producing hyperexcitability (Stanton et al., 1996). The BCM-rule with a sliding threshold of plasticity is commonly also termed 'metaplasticity', i.e. the plasticity of synaptic plasticity (Abraham & Bear, 1996).

Experimental evidence to support the BCM rule has been found in the visual cortex (Kirkwood et al., 1996; Philpot et al., 2001; Philpot et al., 2003) where sensory deprivation alters NMDA receptor subunit composition such that LTD is easier to induce, while prior experience changes NMDA receptor subunit composition and reduces LTD. The BCM-rule has also shown to be valid indirectly in the hippocampus (Whitlock et al., 2006) and in the motor cortex (Rioult-Pedotti et al., 1998, Rioult-Pedotti et al., 2000; Harms et al., 2008) where prior experience occludes further LTP induction; this relationship of plasticity with learning is discussed in greater detail in section 1.5.1.

1.1.4 Homeostatic plasticity

Homeostatic plasticity is a distinct mechanism from metaplasticity but is an indirect consequence of the BCM rule. If LTP was induced, the θ_M would slide to the right making further LTP harder to induce, thus reducing the bidirectionality of synaptic plasticity. A homeostatic mechanism is thus required to realign the θ_M back to a physiological range, and this mechanism is termed homeostatic plasticity.

Homeostatic plasticity is a mechanism by which synaptic efficacy appears to ‘scale’ up after a period of low synaptic activity (and vice versa) (Turrigiano et al., 1998; Leslie et al., 2001) and is related to changes in postsynaptic glutamate receptors (Watt et al., 2000; Wieranga et al., 2005) and postsynaptic ion channels (Misonou et al., 2004). This allows there to be sufficient range for further bidirectional LTP or LTD to be induced (Burrone & Murthy, 2003; Rabinovitch & Segev, 2008) while maintaining the relative weights of the different synaptic inputs to a neuron (Turrigiano et al., 1998). It is important to note

though that the time scale of homeostatic plasticity is in the order of hours, so it is unlikely that it is relevant in rapid acquisition or the early phases of plasticity or learning.

1.1.5 Brain-derived neurotrophic factor (BDNF) and neuroplasticity

The neurotrophin, brain-derived neurotrophic factor (BDNF) has been demonstrated to play a central role in both Hebbian plasticity and this regulatory homeostatic process (Rutherford et al., 1998; Turrigiano & Nelson, 2000; Leslie et al., 2001; Copi et al., 2005). BDNF acts on TrkB post-synaptic receptors to produce LTP without requiring neuronal stimulation (Ying et al., 2002; Messaouodi et al., 2002; Bekinschtein et al., 2008) and when combined with theta burst stimulation facilitates late phase LTP (Pang et al., 2004; Kramár et al., 2004). In addition, chronic bath exposure to BDNF blocks the ‘scaling up’ of synaptic activity during a period of low synaptic activity (Rutherford et al., 1998; Desai et al., 1999).

BDNF makes an attractive candidate for being a regulator of activity-dependent processes like the BCM rule and homeostatic plasticity as it is released in an activity-dependent manner by the dendrites of neurons (Zafra et al., 1991; Wetmore et al., 1994; Lindholm et al., 1994) and there is evidence for synapse-specific release as well (Schinder et al., 2000; Hartmann et al., 2001; Kojima et al., 2001). A review of the molecular biology of BDNF can be found in Lu et al., 2003.

1.2 The study of plasticity in humans

The study of neuroplasticity in humans was initially limited to the study of cultured human neurons or slices from surgical excisions in patients with epilepsy. However when

Anthony Barker and colleagues developed transcranial magnetic stimulation (Barker et al., 1985), this technique spurred newer different types of non-invasive stimulation which allowed the study of neuroplasticity in humans. The mechanism of transcranial magnetic stimulation (TMS) will be discussed first as the principles are central in understanding how neuroplasticity in humans is studied.

1.2.1 Transcranial magnetic stimulation

Transcranial magnetic stimulation uses a coil carrying a rapidly changing electrical current which produces a magnetic field at right angles to the current. If the coil is placed on the scalp, the magnetic field will in turn induce an electrical current in the underlying cortex. If the electrical current is perpendicular to neuronal plasma membranes, this can depolarise the neuron. Thus, a TMS pulse would induce neurons in underlying cortex to discharge an action potential.

TMS to the primary motor cortex produces a motor-evoked potential (MEP) on surface electromyography (EMG) in peripheral muscles that are represented by that region of cortex (Day et al., 1989). An MEP is the summation of the discharge of multiple motor units, and epidural recordings of the pyramidal tract demonstrate that the discharge down the pyramidal tract consists of a D wave and several I waves with more I-waves being recruited with increasing intensity of stimulation (Di Lazzaro et al., 1998; Fig 1.3).

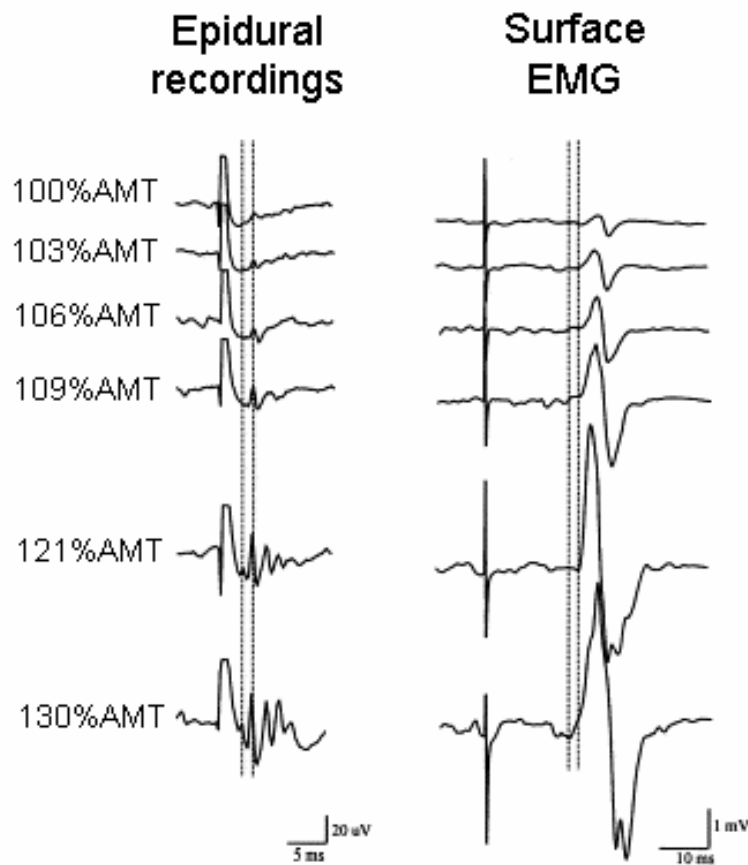


Fig 1.3 Example of epidural volleys and motor-evoked potentials recorded after a single TMS pulse over the primary motor cortex (Adapted from Di Lazzaro et al., 1998)

The prevailing consensus is that the D-wave represents TMS activation of the corticospinal pyramidal neurons while I-waves represent trans-synaptic activation of the pyramidal neuron by excitatory interneurons depolarised by the TMS pulse. One curious feature of I-waves is that successive I-waves are recruited at 1.3-1.5ms periodicity (Di Lazzaro et al., 1998).

As TMS has a high degree of temporal resolution, paired-pulse techniques and techniques combining peripheral electrical stimulation has allowed the measurement of a plethora of intracortical circuits (Table 1.1):

Measurement	Description	Reference
Short-interval intracortical inhibition (SICI)	Subthreshold conditioning TMS pulse 2-3ms before test TMS pulse	Kujirai et al., 1993
Intracortical facilitation (ICF)	Subthreshold conditioning TMS pulse 8-15ms before test TMS pulse	Kujirai et al., 1993
Long-interval intracortical inhibition (LICI)	Suprathreshold conditioning TMS pulse 100-200ms before test TMS pulse	Valls-Solé et al., 1992; Wassermann et al., 1996
Short-latency afferent inhibition (SAI)	Peripheral conditioning electrical stimulation 20-24ms before test TMS pulse	Tokimura et al., 2000
Long-latency afferent inhibition (LAI)	Peripheral conditioning electrical stimulation 50-100ms before test TMS pulse	Sailer et al., 2002
Interhemispheric inhibition (IHI)	Suprathreshold conditioning TMS pulse to contralateral M1 8-40ms before test TMS pulse	Ferbert et al., 1992
Interhemispheric facilitation (IHF)	Near suprathreshold conditioning TMS pulse to contralateral M1 10ms before test TMS pulse	Mochizuki et al., 2004; Baumer et al., 2006
Ipsilateral Premotor inhibition	Near subthreshold conditioning TMS pulse to ipsilateral premotor area 6-8ms before test TMS pulse	Civardi et al., 2001
Interhemispheric premotor inhibition	Near subthreshold conditioning TMS pulse to contralateral premotor area 8-10ms before test TMS pulse	Mochizuki et al., 2004; Baumer et al., 2006
Posterior parietal motor inhibition	Near threshold conditioning TMS pulse to ipsilateral M1 3-10ms before test TMS pulse	Koch et al., 2007

Table 1.1 Summary of the various paired-pulse measurements as recorded from TMS pulses delivered to the hand muscles

Single and paired-pulse TMS allows the measurement of the excitability and synaptic efficacy of excitatory and inhibitory neurons in the motor cortex thereby allowing the measurement of neuroplastic changes *in vivo*.

1.2.2 Repetitive transcranial magnetic stimulation

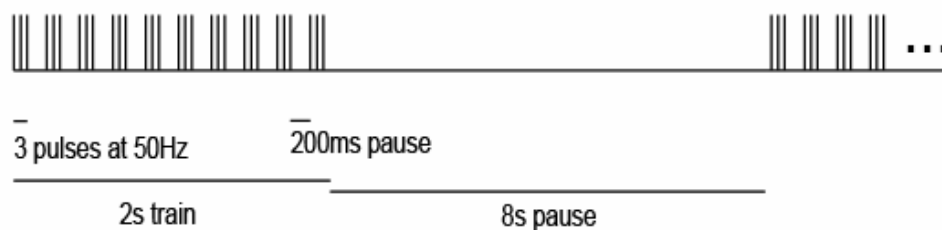
Repetitive transcranial magnetic stimulation (rTMS) delivers trains of TMS pulses to the cortex non-invasively, and unlike single and paired-pulse TMS produces effects which outlast the period of stimulation (Fitzgerald et al., 2006). The chief effect is that it can modify the subsequent MEP amplitude evoked by a single TMS pulse: low-frequency (1Hz) rTMS reduces MEP amplitude (Chen et al., 1997) and high-frequency (>5Hz) rTMS increases MEP amplitude (Pascual-Leone et al., 1994). As these changes are blocked by an NMDA antagonist (Ziemann et al., 1998), it is thought that the changes in MEP amplitudes reflect changes in synaptic efficacy of excitatory interneurons synapsing onto corticospinal pyramidal neurons and this has also been confirmed by epidural recordings of I-wave changes (Di Lazzaro et al., 2008).

1.2.2.1 Theta burst stimulation

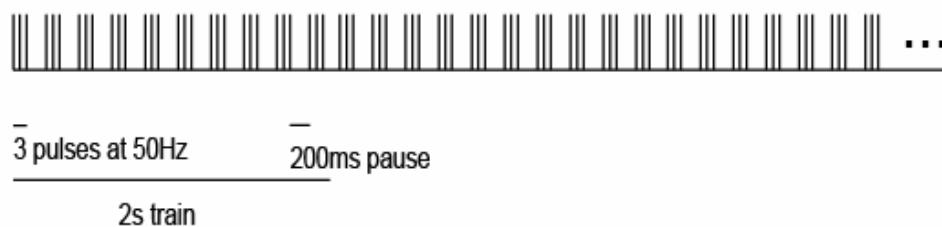
Theta burst stimulation (TBS), is a recent rTMS protocol, which delivers TMS pulses in a high frequency patterned fashion similar to theta burst stimulation used in animal studies of LTP / LTD (Huang et al., 2005). Human TBS consists of bursts of subthreshold TMS pulses delivered at theta-frequency (5Hz). Animal studies have suggested that this is both similar to physiological bursting patterns and optimal for inducing LTP (Larson et al., 1986) as the first burst primes the neurons for plastic changes from subsequent bursts

(Larson & Lynch, 1986; Pacelli et al., 1989). The advantage of this technique is also that the low intensity of stimulation increases the spatial specificity of the TMS delivered and the high frequency reduces the duration of stimulation needed. In the original description of TBS, two forms of TBS were developed: continuous TBS (cTBS) and intermittent TBS (iTBS).

cTBS consists of 200 bursts (consisting of triplet of TMS pulses at 50Hz) delivered at 5Hz (i.e. total of 600 TMS pulses) and iTBS is similar to cTBS except there are pauses of 8 seconds after every 20 bursts (Fig 1.4)



Intermittent theta burst stimulation



Continuous theta burst stimulation

Fig 1.4 Pictorial representation of cTBS and iTBS induction protocols

There are some important caveats to note: TBS produces changes in MEP amplitude despite the stimulation being delivered below the threshold for stimulating excitatory

interneurons or pyramidal neurons that produce MEPs. Additionally, just like some other forms of brain stimulation, the effects on MEPs and intracortical inhibition do not occur immediately after the induction stimulation but build up to peak about 5-10 minutes later (Huang et al., 2005). These caveats need to be taken into account when considering the arguments on the similarity between TBS-induced plasticity and animal models of Hebbian plasticity.

1.2.2.2 I-wave interval rTMS

I-wave interval rTMS is another newer form of rTMS which uses a patterned delivery of TMS pulses. Pairs of TMS pulses with an inter-stimulus interval of 1.5ms (I-wave interval) are delivered at a low frequency; this makes use of the periodicity of I-waves for coincident summation of presynaptic and postsynaptic action potentials to produce progressive enlargement of MEP amplitudes (Thickbroom et al., 2006; Benwell et al., 2006). This is a relatively new technique and the advantage of this technique over other rTMS protocols remains to be determined.

1.2.3 Paired associative stimulation

Paired associative stimulation is another plasticity-inducing TMS protocol that has borrowed on principles from animal models of Hebbian plasticity. This uses the phenomenon of spike-timing-dependent plasticity (Markram et al., 1997): when the discharge of a presynaptic neuron is followed very shortly after by depolarization of the postsynaptic neuron, LTP is produced after only a few paired stimuli, compared to several hundred stimuli with repetitive tetanic stimulation.

In humans, the same phenomenon is produced by paired associative stimulation, PAS (Stefan et al., 2000), where a peripheral electrical nerve stimulus is paired with a TMS pulse to the primary motor cortex of the same body part. The peripheral electrical nerve stimulus produces an afferent volley to the somatosensory cortex (S1) then to the primary motor cortex (M1). This afferent impulse to M1 arrives at the same time as the corticospinal neurons are depolarised by the TMS pulse. The net effect is that after ~90 pairings of electrical and TMS stimulus at about 22ms inter-stimulus interval, MEP amplitude enlarges in a somatotopic fashion (Stefan et al., 2000).

The phenomenon of spike-timing-dependent LTD also occurs with PAS (Wolters et al., 2003): when the TMS pulse precedes the arrival of the peripheral electrical pulse (at inter-stimulus interval of 15ms), MEP amplitudes is reduced.

1.2.4 Transcranial direct current stimulation

Early work done in animals to modulate neuronal resting membrane potentials showed that weak direct currents (DC) can modulate the firing rates of cortical neurons (Creutzfeldt et al., 1962; Purpura & McMurtry, 1965). Transcranial direct current stimulation (TDCS) is a revival of this observation where weak DC current is applied to the scalp and alters underlying cortical excitability as measured by motor-evoked potentials (Nitsche & Paulus, 2000). When the anodal electrode is placed over the primary motor cortex, there is an immediate long-lasting increase in MEP amplitudes and when the cathodal electrode is placed over the primary motor cortex, there is an immediate long-lasting decrease in MEP amplitudes. Both these changes are blocked by an NMDA antagonist, while the voltage-gated sodium-channel blocker, carbamazepine,

only blocked the MEP-facilitating effects of anodal TDCS (Liebetanz et al., 2002) suggesting that changes in synaptic efficacy is the underlying mechanism for the change in MEP amplitudes.

The current consensus is that the weak depolarising current of anodal TDCS shifts the resting membrane potential of postsynaptic neurons such that postsynaptic neurons require less synaptic inputs to produce an action potential, thereby biasing the induction of LTP (Nitsche et al., 2003). The converse applies to the hyperpolarising current of cathodal TDCS.

1.2.5 Direct cortical stimulation

As yet, there are no studies of plasticity in humans after direct cortical stimulation, due to technical feasibility and ethical concerns, although some safety studies of cortical stimulation in stroke patients are encouraging (Brown et al., 2006; Levy et al., 2008).

1.2.6 Similarities between human and animal neuroplasticity models

In humans, motor-evoked potentials (MEPs) are the commonest means of measuring neuroplasticity as changes in this measure are believed to reflect changes in synaptic efficacy of excitatory interneurons onto pyramidal neurons (Ziemann et al., 1998).

Neuroplasticity can be induced by various types of non-invasive stimulation, e.g. repetitive transcranial magnetic stimulation (rTMS), paired associative stimulation (PAS) and transcranial direct current stimulation (TDCS). Some characteristics that the changes produced by these non-invasive stimulation paradigms in humans share with animal models of LTP include:

- 1) Blocking NMDA receptors blocks changes in MEP amplitude (NMDA-dependent) (Ziemann et al., 2001; Stefan et al., 2002; Liebetanz et al., 2002)
- 2) Repetitive transcranial magnetic stimulation increases or decreases MEP amplitude depending on frequency of stimulation (frequency-dependent) (Pascual-Leone et al., 1994; Chen et al., 1997)
- 3) Precise timing of stimuli can produce changes in MEP amplitude in paired-associative stimulation (spike-timing dependent) (Wolters et al., 2003)
- 4) The changes in MEP amplitude have a degree of somatotopy in paired-associative stimulation (Hebbian plasticity) (Stefan et al., 2000)
- 5) Consecutive sessions of PAS produces an effect similar to the BCM rule and metaplasticity (Müller et al., 2007)
- 6) The effect of BDNF polymorphisms in human plasticity (Cheeran et al., 2008)

All this suggests that paradigms of artificially-induced neuroplasticity in humans are very similar to animal models of LTP and LTD. A few caveats which may or may not be significant however should be noted:

- 1) The changes in MEP in some induction protocols do not always occur immediately after induction
- 2) The changes in MEP in most induction protocols last up to an hour at most (with the exception of transcranial direct current stimulation) although animal models of homeostatic plasticity occurs in the order of hours
- 3) High degree of inter-subject and intra-subject variability
- 4) Little evidence currently exists for newer induction protocols like I-wave interval rTMS or theta-burst stimulation

- 5) Changes in the excitability of corticospinal neurons, rather than just the synaptic efficacy of excitatory interneurons synapsing onto corticospinal neurons, can also produce changes in MEP amplitude.

1.3 Drugs in the study of plasticity

The identification of secondary messenger systems involved in classical LTP/ LTD suggests that neurotransmitters that interact with the same molecular pathways can be used to study the modulation of Hebbian plasticity. In this section, the action of some neuropharmacological agents is reviewed.

1.3.1 Noradrenergic drugs

Noradrenergic regulation of LTP in hippocampus (Hopkins & Johnston, 1988) is complex and is dependent on the type of experimental stimulation provided: theta-burst LTP was not affected but LTP induced by lower frequency stimulation was facilitated and induction of LTD was inhibited (Katsuki et al., 1997). These effects in the hippocampus are mediated by both β -adrenoceptors and $\alpha 1$ -adrenoceptors. The role of noradrenergic input in regulating neuroplasticity in other brain regions however is less clear, although recently noradrenergic modulation of LTP of inhibitory synapses in the visual cortex have been described (Yamada et al., 2006).

Neuroplasticity with noradrenergic drugs was considered a likely candidate for modulating human neuroplasticity with the discovery that amphetamine increased motor recovery in primate cortical stroke models (Barbay et al., 2006). In humans, amphetamine

prolonged the effects of TDCS (Nitsche et al., 2004) and noradrenergic agonism enhanced changes in motor representation after practice (Mientzschel & Ziemann 2006).

1.3.2 Dopaminergic drugs

Dopamine plays a role in hippocampal Hebbian plasticity (Kusuki et al., 1997; Swant & Wagner, 2005; Granado et al., 2008) and corticostriatal Hebbian plasticity (Centonze et al., 1999; Pawlak & Kerr, 2008). For corticostriatal plasticity, dopamine appears to be critical for Hebbian plasticity to occur there (Calebresi et al., 2007; Pawlak & Kerr 2008), whereas in the hippocampus dopamine only facilitates LTP/ LTD (Li et al., 2003; Lemon & Manahan-Vaughan, 2006).

Hebbian plasticity in the striatum is complex and involves the interaction of both D1 and D2 dopamine receptors and glutamate receptors on medium-spiny interneurons. The prevailing opinion is that LTD at the corticostriatal synapse requires the activation of both D1 and D2 dopamine receptors (but not NMDA glutamate receptors) (Picconi et al., 2003; Picconi et al., 2008), while LTP at the corticostriatal synapse is NMDA-dependent and requires D1 receptor activation but is inhibited by D2 receptor activation. In addition, chronic administration of levodopa, the precursor molecule to dopamine, which is known to alter the expression of corticostriatal dopamine receptors, produces a loss of corticostriatal plasticity (Picconi et al. 2003). More recent work has further expanded the role of dopamine by showing that dopamine plays a role in ensuring that Hebbian plasticity remains bidirectional (Shen et al., 2008). Thus, it is likely that the patterned release of dopamine by nigrostriatal projections plays a role in governing metaplasticity at the corticostriatal synapse and that disruption of dopamine output in diseases like

Parkinson's disease produces abnormal expressions of plasticity (Calebresi et al., 2007; Shen et al., 2008).

In the neocortex, there are limited studies in animals suggesting a role for dopamine in facilitating both LTD and LTP (Otani et al., 1998; Otani et al., 2003; Matsuda et al., 2006), but it is still unclear if it merely modulates Hebbian plasticity as it does in the hippocampus, or is critical to Hebbian plasticity as in the striatum.

In human studies, LTP-like plasticity is restored by dopamine in Parkinson's disease patients (Morgante et al., 2006), while non-specific dopamine receptor activation with the dopamine agonist pergolide, or enhancement of dopamine release using the dopamine precursor levodopa, produced varying effects depending on the method of brain stimulation (Mientzschel & Ziemann 2006; Kuo et al., 2008; Lang et al., 2008).

1.3.3 Cholinergic drugs

The role of acetylcholine in neuroplasticity was first suggested by the role of cholinergic fibers from the basal forebrain in modulating hippocampal theta rhythm (Teitelbaum et al., 1975; Auerbach & Segal, 1996) and hippocampal LTP. In the hippocampus, muscarine depressed LTP in the CA3 region of the hippocampus (Williams & Johnston, 1988), while in the dentate and CA1 region muscarinic receptor agonism facilitated LTP (Blitzer et al., 1990). Muscarinic receptors also regulate plasticity in the striatum where cholinergic interneurons synapse onto striatal neurons of the indirect pathway and regulate LTD there (Wang et al., 2006; Shen et al., 2008).

In the neocortex, muscarinic receptor blockade inhibits LTP in layer II/III synapses in the primary motor cortex (Hess & Donoghue, 1999) and in the visual cortex (Dringenberg et

al., 2007). There is also evidence that the activation of nicotinic acetylcholine receptors can both modulate NMDA-dependent synaptic plasticity (Ji et al., 2001; Couey et al., 2007) and can also induce LTP independently of the NMDA receptor (Matsuyama et al., 2000; Yamazaki et al., 2005).

Additionally, cholinergic neurons have direct effects on the firing rates of cortical inhibitory interneurons, with segregation of nicotinic and muscarinic receptors on distinct inhibitory interneurons (Xiang et al., 1998; Kruglikov & Rudy, 2008), which some have suggested plays a role in sensory gating and regulating plasticity during the sleep-wake cycle (Steriade & Timofeev, 2003; Lee et al., 2005).

In humans, the plasticity of cortical motor representations associated with practice is blocked by scopolamine (Sawaki et al., 2002) and enhanced by the acetylcholinesterase inhibitor, tacrine (Mientzschel & Ziemann 2006), and plasticity-inducing stimulation in humans are also modulated by the acetylcholinesterase inhibitor, rivastigmine (Kuo et al., 2007).

1.3.4 GABA-ergic drugs

The gating of LTP by GABA inhibition has been appreciated since the early days of the discovery of LTP (Douglas et al., 1982; Wigström & Gustafsson, 1986; Del Cerro et al., 1992), but the pharmacological characterization of GABA receptors has allowed for more detailed study of their role in regulating plasticity. GABA-B autoreceptors activation on the presynaptic inhibitory interneuron have been shown to promote the induction of LTP (Davies et al., 1991; Mott & Lewis 1991), and in animals the LTP-induction protocol,

theta-burst stimulation, is believed to capitalise on this gating phenomenon (Larson & Lynch, 1986; Pacelli et al., 1989; Hess et al., 1996).

In humans, the after-effects rTMS and PAS are inhibited by lorazepam and diazepam (Ziemann et al., 1998; Stefan et al., 2002) while the after-effects of anodal TDCS are also modulated by lorazepam but not cathodal stimulation (Nitsche et al., 2004).

However, animal models of plasticity where activation of GABA-B autoreceptors facilitated the induction of LTP (Davies et al., 1991; Mott & Lewis 1991) was not replicated in the human model of neuroplasticity, paired-associative stimulation (McDonnell et al., 2007). It remains to be determined if this discrepancy also applies to other human neuroplasticity models.

1.3.5 Endocannabinoids

The discovery of endogenous synthesis of substances that bind to cannabinoid receptors (Devane et al., 1992) heralded the discovery of a number of endocannabinoids including anandamide (Devane et al., 1992) and 2-arachidonoyl glycerol (2-AG) (Stella et al., 1997). Depolarised postsynaptic cells synthesise and release these lipid-soluble molecules (Devane & Axelrod, 1994) and the localisation of the CB1 cannabinoid receptor to the axons and presynaptic terminals (Tsou et al., 1992; Mackie et al., 2005; Nyiri et al., 2005) suggest they act as retrograde messengers in synaptic transmission (Wilson & Nicoll, 2001). Activation of the CB1 receptor decreases presynaptic neurotransmitter release (Shen et al., 1996) and endocannabinoids are synthesised during periods of postsynaptic depolarization suggesting an activity-dependent function. The role of endocannabinoids

in synaptic plasticity is diverse and these compounds are found throughout the nervous system (for a more comprehensive review: Mackie, 2008).

It is currently widely accepted that endocannabinoids are the mediator of the phenomenon of depolarisation-induced suppression of inhibition (DSI), where induction of LTP at a glutamatergic synapse also induces reduction in surrounding GABA-ergic inhibitory transmission (Pitler & Alger, 1992; Földy et al., 2005), thereby acting as a metaplastic primer to facilitate further LTP to occur (Chevalleyre et al., 2004; Carlson et al., 2004). The segregation of endocannabinoid signaling onto only a subset of inhibitory synapses (Bacci et al., 2004; Galarreta et al., 2008) is also very suggestive of a complex role played by these molecules.

So far there is no direct evidence for endocannabinoid modulation in human models of neuroplasticity as there are limited pharmacological agents available for use in humans. In an animal model of Parkinson's disease, inhibitors of endocannabinoid degradation rescue some striatal plasticity and improve motor deficits (Kreitzer & Malenka, 2007) making the study of endocannabinoids in human neuroplasticity a promising area of continuing research.

1.3.5 Glutamatergic drugs

The role of AMPA glutamate receptors has become an area of intense interest as it has been suggested that modulation of the activity of these receptors may promote memory encoding. Positive modulation of these receptors promote glutamatergic synaptic transmission by prolonging the opening times of these key glutamatergic receptors (Jin et al., 2005) and thus promote the hippocampal LTP duration and magnitude (Arai et al.,

2004), but the scientific literature is limited on these compounds due to commercial pharmaceutical interest (Cortex Pharmaceuticals and Schering-Plough). The only compound formally tested in the scientific literature is CX516 and although it had some effects on short term memory, no proper tests of human models of neuroplasticity or motor learning have been formed (Wezenberg et al., 2007).

1.4 Motor learning

Conventional classifications of learning and memory distinguish between learning of explicit memories and learning of implicit memories. Motor learning belongs in the category of implicit learning where complex information is learnt without the ability to provide conscious verbal recollection of what has been learned. Perhaps because of this, there are few universally agreed definitions of motor learning and many have grappled with defining it and settled with pragmatic definitions:

“Motor learning does not need to be rigidly defined in order to be effectively studied. Instead it is better thought of as a fuzzy category that includes skill acquisition, motor adaptation, such as prism adaptation, and decision making, that is, the ability to select the correct movement in the proper context. A motor skill is the ability to plan and execute a movement goal.”

- Krakauer, 2006

Meanwhile, others have opted for mechanistic descriptions rather than actual definitions:

“Motor learning takes many forms, including: (1) learning over generations that becomes encoded in the genome, is epigenetically

expressed as instincts and reflexes, and contributes to learned (conditioned) reflexes; (2) learning new skills to augment your inherited motor repertoire, and adapting those skills to maintain performance at a given level; and (3) learning what movements to make and when to make them.”

- Shadmehr & Wise, 2005

For the purposes of this thesis, it is appropriate to use the broadest definition of motor learning: a lasting change in motor performance shaped by prior experience. This encompasses the following definition of learning:

Learning involves changes in behaviour that arise from interaction with the environment and is distinct from maturation, which involves changes that occur independent of such interaction.

- Wolpert et al., 2003

A key feature from these definitions and descriptions of motor learning is that it involves changes in motor performance, but as motor performance (outcome) can be measured in a number of different ways depending on the goal, intrinsic in the definition is the recognition that the optimisation of motor performance is both task-specific and goal-specific. As such, the study of motor learning requires the appreciation of the paradigms used to study motor learning.

1.4.1 Motor learning paradigms

There are a growing number of motor learning paradigms and some common types are reviewed as follows:

1.4.1.1 Sequence learning

Sequence learning or procedural learning was first devised as the serial reaction time task (SRTT) (Nissen & Bullemer, 1987). Subjects performed a repeating series of button presses and the reaction times became progressively faster. Then a different series of button presses was presented and the reaction time would be slower. Thus, the difference between the repeating sequence and a new random sequence allows a measure of learning. There are a number of variants that deal with deficiencies of this task using mixed or probabilistic sequences of button presses, non-spatial colour cues, measurement of learning in the non-performing hand or using more complex movements.

Functional imaging studies have identified underlying brain networks that are associated with this task: dorsolateral prefrontal cortex, supplementary motor area and cerebellum. There are some deficiencies with this paradigm, including the lack of generalisation of this learning and the ecological validity when used as a clinical test (Muslimovic et al., 2007). Nonetheless it is one of the most established and widely used paradigm of motor learning in man.

1.4.1.2 Ballistic motor learning

The classic ballistic motor learning task was devised by Muellbacher et al., 2001, and showed that voluntary repeated thumb abduction progressively increased peak thumb acceleration. The plastic changes required for performance improvement in this task are localised in the primary motor cortex (Muellbacher et al. 2002), and changes in motor representation are also documented (Classen et al. 1998). Additionally, repeated use alone is associated with changes in cortical excitability (Muellbacher et al., 2001; Lotze

et al., 2003; Kaelin-Lang et al., 2005) and functional activation patterns on fMRI (Karni et al., 1998).

Some have termed this a 'practice' or 'use'-dependent effect rather than learning as it is intuitively difficult to see the 'learning' behind this process. However, from a motor system's perspective, the motor system certainly has to acquire new information resulting in lasting performance improvement over time, by 'learning' the combination of agonist and antagonist motor units required to produce the optimal thumb peak acceleration. This suggests that this form of motor learning is a very elementary form of motor learning. This is discussed in greater detail in Chapter 5 of this thesis.

1.4.1.3 Visuomotor transformations

Visuomotor transformation is a broad category of tasks which involve performing a motor task with transformation of the visual sensory feedback (e.g. displacements, rotations, inversions, mirroring and depth distortion), thus combining elements of visual and proprioceptive sensory learning combined with motor learning. Classic tasks include mirror drawing (Corkin, 1968) and rotor pursuit (Ammons, 1951; Ammons et al., 1958). Mirror drawing is self-explanatory but can be difficult to quantify. Rotor pursuit is a continuous motor task where subjects have to track a predictably rotating target (rotor) with the hand (Ammons, 1951). Modern versions of the rotor pursuit task use styluses or computer screens to provide the visual feedback and allow manipulation of visual feedback independent of proprioceptive feedback.

This group of tasks involve a number of secondary motor areas and sensorimotor association areas like premotor cortex, supplementary motor area (SMA), inferior frontal,

dorsolateral prefrontal cortex (DLPFC) and inferior parietal cortex, as well as cerebellum, basal ganglia and primary motor cortex (Halsband & Lange, 2006), but the heterogeneity of the visual and sensory feedback used makes it difficult to compare tasks within this category.

1.4.1.4 Force field adaptation

The force field adaptation task involves manipulating a robot arm in a reaching movement with the robot arm providing resistance, thereby simulating a force field (Shadmehr & Mussa-Ivaldi, 1994). As the force field affects the dynamics of the reaching movement, initially, movement trajectories are grossly distorted but with repeated movements, reaching trajectories resemble more normal movements in free space. This experimental paradigm proposes a system whereby the nervous system gradually builds an internal model of the force field and adapts motor behaviour '*using an intrinsic coordinate system of the sensors and actuators*' (Shadmehr & Mussa-Ivaldi, 1994; Conditt et al., 1997). This paradigm is also related to visuomotor transformations as it requires the mapping of sensory input to motor commands, but requires that at baseline there is already an optimised model of performance. Functional imaging shows that hours after practice, the performance improvement is retained but there is a change in the activation pattern with more premotor, parietal and cerebellar cortices being recruited (Shadmehr & Holcomb, 1997; Nezafat et al., 2001). Interventions have also suggested that this form of learning is not dependent on the primary motor cortex (Baraduc et al., 2004).

This task has been extensively studied and complex computational models have been derived based on experimental data (Shadmehr & Wise, 2005), but this is beyond the scope of this thesis. This experimental paradigm is well-studied in the field of robotics and engineering, but the relevance of this task on human motor control is unclear.

1.4.1.5 Locomotor adaptation

Most motor learning paradigms focus on the upper limbs and hands, and there are limited studies on whether motor learning in the lower limbs or trunk operates on similar principles. A new motor learning paradigm to correct this upper-limb bias is ‘split-belt treadmill walking’. In this task, subjects learn to walk on a treadmill with each lower limb on a different belt such that each lower limb walks at a different rate (Morton & Bastian, 2006). This type of motor learning involves adaptation of the central pattern generators (CPG) in the spinal cord and their descending control, and has revealed separate functional networks controlling different walking patterns (Choi & Bastian 2007). As yet, few research groups have used this experimental paradigm.

1.4.1.6 Classical conditioning

Classical conditioning is a form of motor learning that was discovered serendipitously by the Russian physician, Ivan Pavlov, while studying gastric and salivary function of dogs in the 1890s and 1900s for which he won the Nobel Prize in Physiology and Medicine (Pavlov, 1927). In this work, Pavlov described how a sensory stimulus (conditioned stimulus, CS) can be paired with a stimulus (unconditioned stimulus, UCS) that provokes a reflex motor response (unconditioned response, UR), such that after successive pairings

the conditioned stimulus produces the reflex motor response (conditioned response, CR). Pavlov also described the concept of extinction where the association between the CS and UR disappears if the CS is presented repeatedly in the absence of the UCS. Over the 20th century, this paradigm has been developed and the standard modern paradigm in motor learning is the eyeblink classical conditioning experiment (Gormezano, 1966) which is well-described in a number of mammals (including humans). Repeated CS (short auditory tone) played at a short delay before the UCS (a puff of air to the cornea or an electrical stimulus to the supraorbital nerve) eventually produces CRs: blinks occurring before or in the absence of UCS. Detailed knowledge is known about the circuits, brain regions and molecular processes involved in this paradigm with central roles played by pontine structures, the inferior olives, the cerebellar nuclei (and possibly the cerebellar cortex) (Gerwig et al., 2005; Gerwig et al., 2007; Wada et al., 2007). As decerebrate animals have no problems acquire this conditioning (Jirenhed et al., 2007) and the sparing of this form of motor learning in anterograde amnesia (Clark & Squire 1998), this form of motor learning is considered 'primitive' and relatively isolated in brainstem and cerebellar structures. Classical conditioning paradigms are also used to study other types of learning and memory (e.g. fear conditioning), but these other forms of learning are not the subject of this thesis.

1.4.1.7 Aimed rapid movements

All aimed rapid movements are governed by the psychomotor principle, Fitts' Law, first described by Paul Fitts (Fitts, 1954). When pointing rapidly at a target, there is an inverse relationship between speed and accuracy, and Fitts' Law is expressed mathematically as:

$$MT = a + b ID$$

where MT is movement time, ID is index of difficulty, a and b are coefficients.

$$ID = \log_2 (2A/W)$$

where A is the distance from starting point to centre of target,

and W is the width of the target

Fitts' Law thus demonstrates that there is a speed-accuracy trade-off when performing rapid pointing actions. The slope coefficient, b , is of particular interest to motor learning as it roughly translates into the amount that movement time increases for a given unit of difficult, and repeated practice is associated with a reduction of this coefficient (Kelso, 1984; Schmidt & Less, 2005). Thus some have proposed that a change in the slope (b) reflects acquisition of skill (Cohen, 2008). Of note, older individuals tend to have a higher slope (Welford et al., 1969; Goggin & Meeuwsen, 1992; Welsh et al., 2007) and it is unclear if this is due to central nervous or peripheral mechanical factors. There are some inherent limits to this paradigm: peripheral mechanical factors affect the slope, limiting the use of this measure in disease models where peripheral mechanical factors are affected (e.g. spasticity).

It is also worthwhile noting that some rapid aimed movement tasks combine elements of visuomotor transformation: for example, ramped increases of force based on visual feedback (Muellbacher et al., 2001; Ward et al., 2003) clearly comprises visuomotor transformation but also incorporates a speed-accuracy trade-off if precision is required.

1.4.2 Explicit learning in motor learning

The discovery that bilateral temporal lobectomy in humans produced anterograde amnesia (Scoville & Milner, 1957) and the subsequent discovery that the amnesic H.M. could acquire motor skills (Corkin, 1968) led to the prevailing opinion that there is a segregation of neuronal circuits for explicit declarative knowledge and implicit motor skills. This segregation holds true for a number of motor learning paradigms: visuomotor transformation (Corkin 1968), sequence learning (Reber & Squire 1994; Vandenberghe et al., 2006) and eyeblink classical conditioning at short interstimulus intervals (Woodruff-Pak, 1993; Clark & Squire 1998).

This segregation however does not exclude a role for explicit knowledge or simultaneous explicit learning in modulating implicit motor learning, as there is evidence that in sequence learning there is an interaction (Reber & Squire, 1998; Boyd & Winstein, 2004; Vandenberghe et al., 2006; Brown & Robertson 2007). Thus, appreciating the role of explicit knowledge and motivation is important when designing experimental paradigms for motor learning e.g. probabilistic sequence learning (Wilkinson & Jahanshahi, 2007; Vandenberghe et al., 2006; Song et al., 2007).

1.4.3 Adaptation versus skill learning

Shadmehr & Wise (2005) attempted to sub-classify motor learning paradigms and have provided an important distinction between learning of a new motor skill and motor adaptation:

- (1) Learning a motor skill is expansion of motor repertoire or acquisition of a new motor program with generalisation to other tasks;

- (2) Adaptation is the retuning of an existing motor skill in altered circumstances to maintain performance.

From this it is clear then that the sequence learning and classical conditioning paradigms are paradigms that test learning of a new motor program while force-field adaptation and locomotor adaptation are testing motor adaptations. It is more difficult to classify some motor learning paradigms like visuomotor transformations and ballistic motor learning. Visuomotor transformations appear to resemble adaptation as motor performance is optimised under a different set of dimensions, but being able to ‘mirror draw’ is clearly a new motor skill. Ballistic motor learning resembles adaptation although it is clear that a new motor force vector is being learnt.

Different types of motor learning are obviously dependent on different sensory and motor systems but it is less clear if they operate under similar molecular mechanisms of neuroplasticity. Also, it is important to consider that although these motor learning paradigms dominate the study of motor learning, it is unclear how they relate to more ecologically realistic human motor learning behaviours like learning how to cycle, how to play a violin or neurorehabilitation.

1.4.4 Stages of motor learning

Motor learning is also believed to be composed of several stages, with differing systems involved, first described in 1967 (Fitts & Posner, 1967):

- (1) Verbal-cognitive stage: This is the initial stage with a large cognitive component involving interpretation of instructions and assessment of the goals of the task. This phase is characterised by

large errors and extremely variable performance. This phase is also often termed 'familiarisation' and is dependent on attention and some higher cognition.

- (2) Associative stage: This intermediate stage is also known the 'refining' phase, and is characterised by the gradual decrease in errors and variability, and is believed to be the development of associations of sensory cues with movements that more closely achieves the goals ('sensorimotor mapping'). Most experimental paradigms test performance in this phase.
- (3) Autonomous stage: This final stage is not achieved by all individuals, and is characterised by increasing motor and cognitive efficiency with only modest further decreases in variability.

Different stages of motor learning depend on different cognitive or motor 'modules' so the different stages are also likely to be dependent on different brain regions and different types of neuroplastic responses. This staged time course may also be relevant in the process of consolidation where memories are become more resistant to disruption over time. This has been shown to occur in ballistic motor learning which although initially dependent on the primary motor cortex becomes consolidated after 6 hours (Muellbacher et al., 2002). Most motor learning paradigms focus on the intermediate associative phase but clearly occasionally subjects might be performing in a different stage depending on their prior familiarity. Also for some paradigms (e.g. eyeblink classical conditioning) it is difficult to even perceive such a staged process. This staged nature of motor learning

reflects the dynamic nature of motor learning where different processes and cortical regions play roles at different points of the learning process.

1.4.5 Summary of motor learning

The varied paradigms of motor learning may suggest that there are greater differences than similarities between the learning of different motor tasks. However, mathematical relationships between movement characteristics like kinematics, speed, accuracy and cognitive load, suggest that there are underlying principles in motor behaviour and motor learning and these similarities may reflect fundamental characteristics of the brain physiology and network function. Some of these fundamental characteristics are likely to be dependent on molecular principles of neuroplasticity (e.g. interference, permanence) while other characteristics are likely to reflect the interaction and differential dependency between different brain networks (e.g. consolidation, role of explicit learning).

Motor learning is likely to be an emergent phenomenon from the interaction of multiple brain regions rather than isolated neuroplasticity occurring in synapses in only one cortical or subcortical brain region. However, it is likely that within these multiple brain regions neuroplasticity is also what allows the interaction to remain dynamic. Also, the likely emergent nature of motor learning does not exclude the possibility that molecular and physiological mechanisms set rules and limits to motor learning. Thus, the relationship between motor learning and neuroplasticity will be discussed next.

1.5 The relationship between plasticity and motor learning

1.5.1 Evidence from animal models

It is widely believed that there is a link between neuroplasticity and motor learning.

Martin et al., 2000 contend that for the link between synaptic plasticity and memory formation to be confirmed, several criteria are required:

- 1) Correlation: The behavioural parameters of learning should be correlated with some but not necessarily all of the properties of synaptic plasticity.
- 2) Induction: Learning should be associated with the induction of measurable changes in synaptic efficiency at synapses in appropriate networks of the brain; and the induction of such changes at relevant synapses (were this to be feasible) should result in apparent memories.
- 3) Occlusion: Saturation of synaptic plasticity in a network should destroy the pattern of trace strengths corresponding to established memories and occlude new memory encoding.
- 4) Intervention: Blockade or enhancement of synaptic plasticity, achieved by pharmacological, genetic or other manipulations, should have commensurate effects on learning or memory.
- 5) Erasure: Erasure of synaptic plasticity should, at least shortly after learning, induce forgetting.

In the primary motor cortex of animals, most of these criteria have been fulfilled: motor learning impairs further LTP induction in horizontal connections (Sanes and Donoghue, 2000; Rioult-Pedotti et al., 1998, Rioult-Pedotti et al., 2000) which fulfils the criterion of occlusion. The criterion of correlation is fulfilled by evidence of learning-induced functional cortical reorganisation (Nudo et al., 1996; Kleim et al., 1998; Nudo et al., 2001) and the criterion induction is provided by the most direct evidence to date for LTP

occurring physiologically in animals when LTP was induced in a spike-timing-dependent fashion in the primary motor cortex of freely behaving primates, and produced changes in primate cortical representation of motor movements (Jackson et al. 2006). The evidence for the criterion intervention is provided only indirectly: concurrent cortical stimulation with motor training enhanced performance and facilitated re-emergence of cortical maps (Plautz et al., 2003), improved recovery and structural plasticity can be found with concurrent cortical stimulation and rehabilitation in rats (Adkins-Muir & Jones, 2003), and amphetamine has an enhancing effect on motor training and cortical maps (Barbay et al., 2006). The only criterion left to be fulfilled in animals are erasure. Thus the evidence for the link between LTP and motor learning in animals is very convincing.

1.5.2 Evidence from humans

The proof of causation between models of human neuroplasticity and human motor learning is also incomplete. Changes in physiological parameters of excitability are associated with ballistic motor learning, and this is termed practice-dependent plasticity, (Classen et al., 1998; Muellbacher et al., 2001; Muellbacher et al., 2002). These changes in representation are thought to be due to changes in synaptic efficacy probably involving LTP (Ziemann et al., 2001; Boroojerdi et al., 2001), analogous to animal models of practice-dependent plasticity (Sanes & Donoghue, 2000; Rioult-Pedotti et al., 1998, Rioult-Pedotti et al., 2000). These changes are also accompanied by an increase in functional MRI (fMRI) blood oxygen level-dependent (BOLD) signal indicative of increased neural activity (Lotze et al., 2003). Changes in representation and BOLD signal are believed to reflect changes in synaptic efficacy probably involving LTP (Ziemann et

al., 2001; Boroojerdi et al., 2001b), analogous to animal models (Sanes and Donoghue, 2000; Rioult-Pedotti et al., 1998, Rioult-Pedotti et al., 2000). Thus, there is ample evidence to fulfill the criterion of intervention.

Inhibition and disinhibition of practice-dependent plasticity by pharmacologically inhibiting or disinhibiting synaptic transmission and neuroplasticity is associated with poorer rates of motor learning (Donchin et al., 2002; Ziemann et al., 2006) providing evidence of correlation.

There is ample evidence for occlusion of motor learning and human neuroplasticity: Ziemann et al., 2004 have investigated how different paradigms interact with each other and have shown that prior ballistic motor learning interacts with subsequent artificial induction of plasticity by rTMS. This suggests that ballistic motor learning and rTMS-induced plasticity are likely to be interrelated. Stefan et al., 2006 also showed PAS occlusion after force adaptation motor training. The criterion of erasure has been fulfilled by evidence that performance improvements from ballistic motor learning are inhibited when inhibitory 1Hz rTMS is delivered to the primary motor cortex shortly after motor training (Muellbacher et al., 2002).

In humans, there is only limited evidence for induction. One study showed that sequence learning could be enhanced by anodal TDCS (Nitsche et al., 2003) but there has been less success with other paradigms (Agostino et al., 2007). The criterion of induction is difficult to experimentally implement: artificial induction of neuroplasticity alone is unlikely to encode useful information as effective motor learning is likely to require the encoding of information into multiple brain networks. The need for multiple networks is demonstrated by a study showing how motor practice alters MEPs and sensorimotor

organisation while paired-associative stimulation (a paradigm of artificial stimulation) only affects MEPs (Rosenkranz & Rothwell, 2006). This is also supported by the fact that artificially inducing plasticity in the primary motor cortex produces very subtle behavioural changes while by definition most motor learning paradigms show much more obvious behavioural changes (Gerloff et al., 1998; Muellbacher et al., 2000; Baraduc et al., 2004).

The relationship between human neuroplasticity and motor learning is made more complex by the variety of motor learning paradigms available and these different paradigms are dependent on different systems providing different contributions depending on the paradigm.

1.6 Motor learning and plasticity in disease

The study of how diseases of the nervous system affect motor learning and plasticity provides some clues to the structures and processes that support these phenomena, and a few diseases are reviewed as follows:

1.6.1 Stroke

For the purposes of this thesis, the focus will be on strokes affecting the motor function of the limbs, and thus will predominantly focus on middle cerebral artery strokes affecting the cortex and/or related subcortical structures (e.g. internal capsule, basal ganglia, pyramidal tract). Cerebellar strokes are discussed in greater detail in section 1.6.3. Cerebrovascular insults to the primary motor cortex and corticospinal tract are associated with the reorganisation of brain regions occurring over many months and years (Ward et

al., 2003; Kwakkel et al., 2003; Kwakkel et al., 2006; Krakauer et al., 2007). This reorganisation process is believed to involve the resolution of oedema after the insult (Kwakkel et al., 2003; Kwakkel et al., 2006; Krakauer et al., 2007) and neuroplastic changes (Nudo et al., 1996; Nudo et al., 2001; Ward & Cohen, 2004; Krakauer et al., 2007), with the former predominating in the acute and subacute phase and the latter predominating in the subacute and chronic phase (Kwakkel et al., 2003; Kwakkel et al., 2006).

Functional imaging studies have shown that initially there is increased activation of undamaged secondary motor areas after the stroke, and focusing of these widespread activation patterns to fewer areas during functional recovery (Ward et al., 2003).

Additionally, it has also been shown that certain activation patterns on fMRI are associated with poorer outcome: activation of contralateral motor cortices are associated with poorer outcome (Ward et al., 2003; Ward et al., 2004). It has been proposed that there is a hierarchy of functional architecture with the function of the damaged primary motor cortex being taken over by the ipsilesional premotor cortex preferentially, and then the contralesional premotor cortex (Johansen-Berg et al., 2002; Fridman et al., 2004; Ward et al., 2007; Swayne et al., 2008).

1.6.1.1 Stroke recovery through neuroplasticity and motor learning

The process by which this reorganisation occurs is unclear although neuroplasticity (i.e. Hebbian processes or structural plasticity) is the leading candidate mechanism.

Certainly the remaining ipsilesional corticospinal output as measured by MEP recruitment curves and thresholds in the acute period is associated with good recovery

(Cicinelli et al., 1997; Traversa et al., 2000; Swayne et al., 2008), but this clearly measures the scale of the insult rather than the process by which recovery occurs. There is a reduction in intracortical inhibition (i.e. disinhibition) predominating in the affected hemisphere acutely, and this disinhibition resolves in those with good functional recovery (Manganotti et al., 2002). This was replicated in a longitudinal study in stroke which showed that the degree of intracortical disinhibition was negatively correlated with functional status at 3 months (Swayne et al., 2008). The authors theorised that there are several phases to the recovery process with the motor system being reliant on pre-stroke architecture in the acute phase, an adaptive disinhibition response at 3 months allowing distant secondary areas to optimise performance, followed by a chronic phase where distant secondary areas are no longer dependent on primary motor cortical disinhibition. This is consistent with views that intracortical disinhibition primes the motor cortex to neuroplasticity inducing protocols in humans (Ziemann et al., 1998) and in animals (Jacobs & Donoghue, 1991). As yet, there is no direct evidence that patients with stroke in the subacute phase are more plastic although there is some evidence in animal models of stroke (Biernaskie et al., 2004).

The cortical reorganisation after stroke is likely to require shaping to useful representations and it is widely held that physiotherapy and rehabilitation in stroke units provide this by encouraging 'motor learning' (Carr & Shepherd, 2000; Krakauer, 2006). The evidence for this is surprisingly limited: better functional status in chronic stroke is associated with better motor sequence learning (Boyd et al., 2001; Boyd et al., 2007) and force field adaptation (Takahashi & Reinkensmeyer 2003), but these studies are performed in the chronic phase of stroke and it is not clear that well-recovered patients

have achieved their level of performance through preserved motor learning or if preserved motor learning is just an epiphenomenon of a well-recovered patient. More work will need to be done to establish this intuitive link.

1.6.1.2 The ‘hemispheric rivalry’ hypothesis

There is some evidence that the unlesioned primary motor cortex has a negative influence on motor performance after stroke; this is termed the ‘hemispheric rivalry’ hypothesis.

The first suggestion of this occurred when it was found that while the damaged primary motor cortex was hypoexcitable after a stroke, the undamaged primary motor cortex was disinhibited after stroke (Cicinelli et al., 2003; Werhahn et al., 2003; Murase et al., 2004; Ward & Cohen, 2004). The identification of transcallosal inhibitory pathways suggested that the undamaged primary motor cortex suppressed the excitability of the damaged primary motor cortex, thereby providing this negative influence (Murase et al., 2004).

This has prompted many studies on using rTMS or TDCS to suppress the excitability of the unaffected hemisphere and enhance the excitability of the affected hemisphere (Takeuchi et al., 2004; Ward & Cohen, 2004; Mansur et al., 2005; Hummel & Cohen, 2005; Kim et al., 2006; Fregni et al., 2006; Baggio et al., 2006; Talelli et al., 2007; Takeuchi et al., 2008; Nowak et al., 2008). Additionally, Di Lazzaro et al., (2006) reported an interesting single case of hemichorea secondary to stroke dramatically responsive to cTBS to the primary motor cortex indicating that neuroplastic interventions could be tailored to the condition. Whatever the case, if brain stimulation is intended for clinical use, it is hoped that the increase in excitability might allow motor learning to occur, but to date no studies have followed patients up long-term.

The physiotherapy protocol, constraint-induced modified therapy (CIMT) which has recently been shown to be effective in enhancing stroke recovery (Wolf et al., 2006; Liepert et al., 2006; Dahl et al., 2008; Gauthier et al., 2008) is thought to be another way of modulating this rivalry. Restriction of the movements of the unaffected arm is associated with less disinhibition of SICI in the affected hemisphere (Liepert et al., 2006), more gray matter volume (Gauthier et al., 2008) and enlarged cortical representations (Boake et al., 2007), although there is no evidence that transcallosal inhibition mediates this mechanism as yet.

1.6.2 Parkinson's Disease

The nigrostriatal degeneration that typifies Parkinson's disease results in dopaminergic deficiency in the striatum and thus impaired corticostriatal plasticity (discussed in section 1.3.2), but there is also some evidence of impairments in cortical plasticity.

Cortical plasticity as measured by PAS was impaired in Parkinson's disease patients (Ueki et al., 2006) and was restored by levodopa (Morgante et al., 2006). However another study showed that the MEP changes did occur with Parkinson's Disease but were less focal while off dopaminergic medication (Bagnato et al., 2006). The discrepancy may arise from the fact that these studies were performed on patients exposed to dopaminergic therapy for some time, and this is known to affect the dopaminergic receptor expression as well as predispose to dyskinesias. rTMS to the premotor cortex of newly diagnosed Parkinson's disease patients produced effects that lasted longer compared to chronic patients (Buhmann et al., 2004), suggesting that future studies may have to focus on newly diagnosed patients.

It is well-established that motor learning is impaired in Parkinson's Disease: although patients were able to improve their reaction times in sequence motor learning, they required greater number of repetitions to acquire the same level of performance as age-matched controls and this has been demonstrated in a number of studies (Ferraro 1993; Pascual-Leone et al., 1993; Jackson et al., 1995; Sommer et al., 1999; Brown et al., 2003; Wilkinson et al., 2007). An intriguing theory is that the dopaminergic deficiency in the striatum of Parkinson's Disease patients results in insufficient 'motivation' to increase movement speed (Mazzoni et al., 2007), thus the impairments in sequence motor learning is related to a lack of a 'reward' signal. Nonetheless, the clinical significance of this impairment is unclear as the impairment was not correlated with functional scales and only correlated with disability scores (Muslimovic et al., 2007).

These motor learning impairments do not translate to other paradigms: patients with Parkinson's Disease have no problems with eyeblink classical conditioning (Sommer et al., 1999). Insufficient evidence exists for motor learning impairments in other conditions.

The modulation of cortical excitability and plasticity has also been proposed as a possible treatment modality for Parkinson's Disease (Edwards et al., 2008) and numerous trials have confirmed that there are some modest benefits to be gained from this form of treatment (Siebner et al., 1999; Khedr et al., 2006; Lomarev et al., 2006; Hamada et al., 2008).

1.6.2.1 Levodopa-induced dyskinesias

Chronic administration of levodopa is associated with the development of dyskinesia and it has been postulated that this is due to altered plasticity from chronic dopaminergic stimulation (Picconi et al., 2005; Pisani et al., 2005). Evidence that neuroplasticity is altered by dopaminergic medication is provided by the study of Morgante et al., 2006 which demonstrates that levodopa medication fails to restore plasticity in Parkinson's Disease patients with levodopa-induced dyskinesias.

Modulation of neuroplasticity by 5Hz rTMS of the SMA have also been shown to be beneficial (Koch et al., 2005; Brusa et al., 2006; Edwards et al., 2008) and this requires further larger studies.

1.6.3 Cerebellar disease

Cerebellar disease is associated with impairments of motor coordination and control, typified by abnormal kinematics in single and multi-joint movements of ballistic or reaching movements with a prolonged acceleration phase, a delayed deceleration phase and terminal tremor (Berardelli et al., 1996; Day et al., 1998; Diedrichsen et al., 2007).

The role the cerebellum plays in motor learning though was first demonstrated by impairments in classical Pavlovian nictitating membrane (eyelid) conditioning in rabbit (McCormick et al., 1982; McCormick & Thompson, 1984), identifying the dentate-interpositus nucleus as a vital structure in animals. This has been replicated in humans and studies of cerebellar stroke patients exhibiting this impairment have suggested the anterior cerebellar cortex to be a region where this motor learning occurs (Gerwig et al., 2003, Gerwig et al., 2005, Gerwig et al., 2007).

Another form of motor learning dependent on the cerebellum is sequence learning.

Patients with cerebellar disease had little to no improvements in reaction time in the hand ipsilateral to the cerebellar lesion (Pascual-Leone et al., 1993; Molinari et al., 1997; Gómez-Beldarrain et al., 1998; Torriero et al., 2007).

Clearly plasticity within the cerebellum may be affected by any disease process affecting the cerebellum. For example, in Fragile X syndrome there is evidence in animals and in humans of impairments of cerebellar plasticity due to inactivation of the FMR1 gene (Koekkoek et al., 2005) but neuroplasticity in other brain regions appear to be unaffected by cerebellar disease with normal cortical plasticity to paired-associative stimulation in patients with spinocerebellar ataxia 6 (Teo et al., 2008).

1.6.4 Dystonia

Primary dystonia is a disorder of abnormal muscle tone without any other neurological disorder, and is believed to be related to abnormality in basal ganglia functioning (Berardelli et al., 2002; Breakefield et al., 2008) and hyperexcitability of cortical and brainstem interneurons (Sohn et al., 2004; Butefisch et al., 2005; Tisch et al., 2006; Huang et al., 2006; Tamura et al., 2008).

Plasticity have been found to be abnormal in a number of dystonic conditions: abnormal response to facilitatory and inhibitory PAS in primary hand dystonia lasting longer and with less somatotopy (Quartarone et al., 2003; Weise et al., 2006), abnormal response to cTBS in manifesting and non-manifesting carriers of the DYT1 gene (Edwards et al., 2006), abnormal responses to 1Hz rTMS in primary hand dystonia (Quartarone et al., 2005; Baumer et al., 2007); and abnormal plasticity in the blink reflex circuit in

blepharospasm (Quartarone et al., 2006). Additionally, this abnormal plasticity also extends to body parts unaffected by the dystonia (Quartarone et al., 2008) prompting the hypothesis that the primary abnormality in dystonia is a problem of plasticity (Classen, 2003; Quartarone et al., 2008).

Primary adult-onset dystonia is associated with rehearsed skilled motor tasks, thereby producing a proliferation of manifestations (e.g. writer's cramp, musician's dystonia, golfer's yip), and this is suggestive of a role for motor learning in this condition. Thus it is surprising that the literature on motor learning in primary dystonia is sparse, and only motor sequence learning has been reported in these patients. Motor sequence learning is impaired in carriers of DYT1 gene (Ghilardi et al., 2003), with increasing dependency on the cerebellum even in non-manifesting carriers (Carbon et al., 2008). Clearly additional work will need to be done to explore motor learning in patients with primary dystonia.

1.6.5. Huntington's disease

Huntington's disease is a neurodegenerative disorder characterised by striatal degeneration related to the *huntingtin* protein. The striatal degeneration is focused predominantly on the striatal medium spiny neurons that receive dopaminergic and glutamatergic input from the cortex. Animal models of Huntington's disease display aberrant hippocampal and cortical synaptic plasticity (Usdin et al., 1999; Cummings et al., 2006), which can be restored by BDNF (Lynch et al., 2007). Likewise, human models of cortical plasticity also demonstrate impairment in responses to PAS (Crupi et al., 2008) and bursts of high-frequency rTMS (Lorenzani et al., 2006). A pilot study has also

suggested that 1Hz rTMS reduces severity of chorea in Huntington's disease for about 30 minutes (Brusa et al., 2005).

Motor learning in Huntington's disease is limited to demonstrating impairment of sequence learning (Knopman & Nissen, 1991) which is unsurprising considering the dependence of this motor learning task on striatal circuits (Wilkinson & Jahanshahi 2007).

1.6.6 Alzheimer's Disease

Alzheimer's disease is a diffuse neurodegenerative process which is typified by loss of declarative and episodic memory, and there is evidence that the beta-amyloid protein which defines this condition impairs LTP, enhances LTD and reduces dendritic structural plasticity in the rat hippocampus (Shankar et al., 2008). It is also established that in most paradigms of motor learning are spared, e.g. sequence motor learning (Willingham et al., 1997), mirror drawing (Rouleau et al., 2002) and rotor pursuit (Deweert et al., 1994; Jacobs et al., 1999). However, cortical measures of excitability and integration demonstrate impairments in short-latency afferent inhibition (a parameter affected by reduced muscarinic receptor activation), and a recent study has also demonstrated that 5Hz rTMS fails to facilitate MEPs in patients (Inghilleri et al., 2006). It is certainly possible that in the early stage of the disease, although there are impairments in motor cortical plasticity related to diffuse neurodegeneration, motor learning is still relatively spared and motor learning paradigms are not sensitive enough to detect this. Whatever the case, insufficient work has been done on Alzheimer's Disease at this stage to arrive at any definite conclusions.

1.6.7 Relevance of motor learning to neurorehabilitation

It is widely held that neurorehabilitation is a learning process; motor recovery from stroke depends on re-learning lost skills and learning new compensatory movements (Greenwood 2004; Greenwood & Ward 2007). Certainly it seems intuitive that neurorehabilitation involves motor learning, as patients practice movements showing accompanying motor recovery.

Longitudinal studies of stroke suggest that motor recovery proceeds through a series of stereotypical stages over the first 6 months post-stroke (Kwakkel et al., 2006). Even despite heterogeneity of stroke severity, a logistic regression model based on functional state in the 1st week makes a reasonable prediction of recovery at 6 months accounting for 50% (Kwakkel et al., 2003) to 89% (Prabhakaran et al., 2008) of the variance of the outcome, suggesting that there is a spontaneous process of recovery which is independent of neurorehabilitation and motor learning, and is likely to be related to restoration of activity in the ischemic penumbra, resolution of diaschisis and reduction of cerebral oedema. The remaining variance appears relatively small, offering a pessimistic view of the benefits of neurorehabilitation.

High intensity neurorehabilitation in the first 6 months after stroke produced more rapid improvements in the first 6 months but had similar outcomes after 1 year (Sunderland et al., 1994; Kwakkel et al., 2002). Although at first glance pessimistic, evidence showing lasting benefit from specialised stroke units early in the condition (Indredavik et al., 1999) suggests that duration or intensity is not the issue but quality of the neurorehabilitation.

Thus the apparent small variance of outcome left accountable after spontaneous recovery may be due to insufficient knowledge about how to provide high quality neurorehabilitation that specifically targets the deficits. Thus, neurorehabilitation therapies based on motor learning principles provide a framework to improve outcomes and a number of such therapies have been developed: constraint-induced movement therapy (CIMT) discussed previously (Wolf et al., 2006), EMG-triggered neuromuscular stimulation (Bolton et al., 2004), arm ability training (Platz et al., 2001), robotic therapy (Fasoli et al., 2003; Ferraro et al., 2003) and virtual reality interfaces (Holden, 2005). Certainly the successful trial of CIMT is encouraging and provides a template for how to translate motor learning theories into clinical applications (Academy of Medical Sciences, 2004; Cumberland Consensus Statement, 2008).

1.7 Goal of this thesis

The goal of this thesis is to attempt to study the modulation of neuroplasticity, using pharmacological agents and non-invasive brain stimulation and to describe how this links with motor learning in humans. The use of pharmacological agents is particularly relevant clinically as various drugs have been suggested as candidates to enhance neurorehabilitation (Goldstein, 2000; Goldstein, 2006) and neuroplasticity (Ziemann et al., 2006). The use of non-invasive brain stimulation to modulate excitability and/ or plasticity to thereby affect motor learning in patients and normal humans is also attractive (Nitsche et al., 2003; Takeuchi et al., 2005; Carey et al., 2006; Takeuchi et al., 2008).

This thesis presents 6 studies of neuroplasticity and motor learning focusing on different aspects in this wide field:

Chapter 3 will study the effect of NMDA agonism on theta burst stimulation. The research question was to determine if theta burst rTMS (like other forms of artificially-induced plasticity) is also dependent on NMDA receptors.

Chapter 4 will study the role of various neurotransmitter systems on theta burst stimulation. The research question was to determine if the after-effects of theta burst rTMS could be modulated by various neuromodulators.

Chapter 5 will study the effect of theta burst stimulation on ballistic motor learning. The research question was to determine if theta burst stimulation had any effect on ballistic motor learning and to try to elucidate possible mechanisms for this effect.

Chapter 6 will study the role of different intracortical circuits in motor plasticity. The research question would be to determine which inhibitory intracortical circuits regulate motor plasticity.

Chapter 7 will study the intracortical circuits that modulate transcallosal inhibition and their plasticity. The research question was whether transcallosal output has similar properties to corticospinal output and whether this has a significant influence on the primary motor cortex.

Chapter 8 will study the effect of theta burst stimulation on sequence learning. The research question was whether sequence learning could be modulated by theta burst rTMS and whether any effect was location-specific.

Chapter 2

General Methods

The development of transcranial magnetic stimulation (TMS) and repetitive transcranial magnetic stimulation (rTMS) has allowed the experimental study and manipulation of cortical excitability and plasticity in humans non-invasively. This chapter briefly describes the methods used throughout this thesis.

2.1 Subjects

Subjects were recruited for each study in each chapter independently. Written informed consent was obtained after the subject was provided with an information sheet. The studies, information sheets and protocols were reviewed and approved by the National Hospital for Neurology and Neurosurgery (NHNN) and University College London Hospital (UCLH) research ethics committee. For the use of medicinal compounds in these studies, The Medicines and Healthcare products Regulatory Authority (MHRA) confirmed that these experiments did not constitute a clinical trial. All experiments conformed to the standards set by the Declaration of Helsinki, and subjects were allowed to drop out of the study at any point.

In total, 2 subjects dropped out of all the studies performed and their data were not included in the analysis. The reasons for dropping out were: loss of contact as the subject had left the country, and scalp discomfort from TMS when starting the experiment.

2.2 Electromyography (EMG)

EMG was recorded with silver disc surface electrodes were placed in a tendon-belly montage. Unless otherwise stated, in most experiments the first dorsal interosseus (FDI) muscle was used. The negative electrode was placed over the bulk of the FDI muscle and

the positive electrode over the first metacarpophalangeal joint. The ground electrode was placed at the dorsum of the wrist.

EMG signals were amplified and filtered (20 Hz to 1 kHz) with a D360 amplifier (Digitimer Limited, Welwyn Garden City, UK). The signals were sampled at 5000 Hz, digitised using a laboratory interface (Power1401, Cambridge Electronics Design (CED), Cambridge, UK) and stored on a personal computer for display and later off-line data analysis. Each recording epoch at least 400 ms, of which at least 100 ms preceded the TMS. Trials with muscle activity in the time preceding the TMS was discarded.

2.3 Transcranial magnetic stimulation (TMS)

Transcranial magnetic stimulation was performed with a pair of linked Magstim 200² Bistim magnetic stimulators or a single Magstim 200 magnetic stimulator (Magstim Company, Whitland, Dyfed, UK) depending on whether paired-pulse stimulation was necessary. The magnetic stimuli produced a monophasic pulse with a rise-time of approximately 100µs, decaying back to zero over approximately 0.8ms. The coil was placed tangentially to the scalp with the handle pointing postero-laterally at a 45° angle to the sagittal plane inducing a posterior-anterior current in the brain. This orientation was chosen based on the findings that the lowest motor threshold is achieved when the induced electrical current flows approximately perpendicular to the line of the central sulcus.

We determined the optimal position for activation of the FDI muscles by moving the coil in 0.5 cm steps around the presumed motor hand area of the motor cortex of both hemispheres. The sites where stimuli of slightly suprathreshold intensity consistently

produced the largest MEPs in corresponding FDI muscle (referred to as “motor hot spot”; M1) were marked with a red marker pen by drawing a crescent line following the anterior bifurcation of the coil and a straight line indicating the orientation of the coil.

Resting motor threshold (RMT) was defined as the minimum stimulus intensity that produced a MEP of at least 50 μ V in 5 out of 10 consecutive trials. Active motor threshold (AMT) was determined during voluntary tonic contraction at 5-10% of maximum voluntary contraction (MVC) and was defined as the minimum intensity which produced a MEP of at least 200 μ V at least three out of five consecutive trials. Motor thresholds were expressed as a percentage of maximum stimulator output (MSO).

2.4 Repetitive transcranial magnetic stimulation (rTMS)

Focal rTMS was applied over the left M1 with a Magstim Rapid stimulator and a flat figure-of-eight coil with mean loop diameter of 9 cm. The magnetic stimulus had a biphasic waveform with a pulse width of approximately 300 μ s. The handle of the coil pointed backwards and laterally at a 45° angle from the midline. The coil was placed tangentially to the scalp in the same position used for the single TMS. All rTMS protocols were in accordance with published safety recommendations (Wassermann 1998).

For most studies (Chapter 3, 4, 5 and 8), theta-burst stimulation was used (Huang et al., 2005). This protocol can consist of either an inhibitory continuous protocol consisting of a burst of 3 stimuli at 50Hz (a theta burst) repeated every 200ms for a total of 600 stimuli (inhibitory TBS) or an excitatory intermittent protocol consisting of an 8 second pause

between every 10 theta bursts (excitatory TBS). Each stimulus was delivered at 80% of the individual's active motor threshold over the hand area of the motor cortex.

For chapter 7, conventional repetitive transcranial magnetic stimulation (rTMS) at 5 Hz was delivered at 90%RMT split into two conditioning trains of 300 stimuli each with a pause of 1 minute. This protocol was chosen as inhibitory intracortical circuits was the predominant focus of the study and Quartarone et al. (2005) demonstrated that this protocol affects the excitability of the inhibitory intracortical circuits without significantly affecting the corticospinal excitability.

2.5 Behavioural measures

In chapter 5 and 6, motor learning was assessed using a simple ballistic motor task as previously described (Muellbacher et al., 2001; Muellbacher et al., 2002) while in chapter 8, sequence learning was assessed using a variant of the serial reaction time task (SRTT). Details of the tasks are described in the relevant chapters.

2.6 EMG data analysis

The primary measure of EMG signal in all studies was peak-to-peak amplitude of MEP of individual trials. This was measured using a customized script for Signal software. For paired-pulse and afferent stimulation measurements, peak-to-peak amplitude of conditioned MEP was normalised against peak-to-peak amplitude of test MEP as represented as a percentage. This is described in greater detail in Chapter 6. For analysis of the ipsilateral silent period, the method described by Trompetto et al. 2004 was used. A more complete description is described in Chapter 7.

2.7 Statistical analysis

All statistical procedures were conducted using the statistical package, SPSS 12.0 (SPSS for Windows 12.0 Chicago: SPSS; 2004). Numerical data are mean \pm SD unless otherwise stated. Significance for all procedures was set at a level of 0.05. Details of the various t-tests, analysis of variance (ANOVA) and correlations are described in the relevant chapters.

In Chapter 5, the circular statistics software Oriana (Oriana for Windows, Kovach Computing Services, Anglesey, Wales) was used. The Rayleigh test was first performed on the movement vectors in order to verify that they were not circularly uniform. The concentration parameter (κ) was derived from the TMS-evoked movement vectors. κ is a measure of the directionality of the distribution (Fisher, 1993) for which a value of 0 would represent no vector directionality (a distribution resembling a perfect circle), and thus maximal motor output variability. As κ is a non-linear parameter, it was transformed with log10 and a mean calculated for graphical representation. The non-parametric Wilcoxon paired signed ranks test was used to test for significant differences.

Chapter 3

NMDA agonism and rTMS-induced plasticity

Work described in this chapter has been previously published:

Teo JT, Swayne OB, Rothwell JC. Further evidence for NMDA-dependence of the after-effects of human theta burst stimulation. Clin Neurophysiol. 2007 Jul; 118(7):1649-51.

3.1 Introduction

The activation of postsynaptic N-methyl-D-aspartate (NMDA) receptors is one of the central characteristics of the classical postsynaptic model of LTP and LTD: the induction protocol (with high-frequency stimulation) activates postsynaptic NMDA receptors allowing calcium influx which lead to long-term changes in synaptic strength (MacDermott et al., 1986; Larson et al., 1986; Larson et al., 1988).

Pharmacological blockade of TDCS (Liebetanz et al., 2002) and PAS by the NMDA antagonist dextromethorphan (Stefan et al., 2002) supports the idea that these two paradigms are dependent on LTP.

Pharmacological blockade of the after-effects of TBS has very recently been shown with the NMDA non-competitive antagonist, memantine (Huang et al., 2007). This provides strong evidence that TBS is an NMDA-dependent phenomenon. The question thus arises whether pharmacological activation of the NMDA receptors would alter the effect of TBS. To date, there is only one study which shows an effect of NMDA agonist activity on non-invasive stimulation. Nitsche et al. have shown that the partial NMDA receptor agonist D-cycloserine prolongs the duration of the excitatory effect of anodal TDCS without having any effect on the inhibitory effect of cathodal TDCS (Nitsche et al., 2004a).

The purpose of this study is to study the effect of the NMDA partial agonist D-cycloserine on the effects of TBS. We hypothesised that if the effects are mediated in a straightforward manner by an NMDA-dependent process such as LTP then they should be enhanced by the drug. D-cycloserine was chosen as it is one of the few

NMDA partial agonists available for safe use in humans and to allow a comparison with the effects seen in TDCS (Nitsche et al; 2004a).

3.2 Study design

The study was designed as a double-blind placebo-controlled within-subjects design and recruited with written informed consent. The studies, information sheets and protocols were reviewed and approved by the National Hospital for Neurology and Neurosurgery (NHNN) and University College London Hospital (UCLH) research ethics committee, and conformed with the Declaration of Helsinki.

6 subjects (2 females, 4 males; 30.5 ± 4.4 SD years) were recruited. Each subject participated for 2 sessions and was pseudo-randomised and counterbalanced to receive either 2x50mg of D-cycloserine capsules or 2x50mg of placebo (ascorbic acid) capsules at each session. Sessions were at least 1 week apart to avoid carry-over effects. TBS consisted of bursts containing 3 pulses at 50 Hz at an intensity of 80% AMT repeated at 200 ms intervals (i.e. at theta-bursts of 5 Hz) and given in an intermittent theta burst stimulation pattern (iTBS): a 2 s train of TBS repeated every 10 s for 20 repetitions. This pattern is identical to that used by Huang et al (2005) who showed it to be excitatory, producing increased MEP amplitudes lasting up to 20 minutes. The drug was taken 2 hours before theta-burst stimulation so that it coincided with peak plasma concentration (van Berckel et al., 1997). 30 minutes before receiving theta-burst stimulation, the 'motor hotspot' was identified and RMT and AMT were measured. The intensity of stimulation to elicit an MEP amplitude of approximately 1-mV was set and then a baseline measure of MEPs was recorded consisting of 15 MEP trials. Immediately after, intermittent TBS (iTBS) was delivered to the motor hotspot. MEPs were then recorded using the same intensity of

stimulation as baseline in blocks of 15 trials at various time points after completion of iTBS: 1, 10, 20, 30 and 60 minutes. Subjects were permitted to leave the laboratory for a break after the 30 minute measurement.

3.3 Drug

D-cycloserine is a partial agonist at the glycine-binding site of the NMDA receptor. At low doses (50-250mg) it acts as an agonist, but at higher doses (>500mg) it acts as an antagonist (Watanabe et al., 1992). Thus, the correct dosing of the drug is essential. We chose 100mg as the dose for this study as it has been shown in other non-invasive brain stimulation study to be sufficient to produce a pharmacological agonist effect (Nitsche et al., 2004a). Two 50mg D-cycloserine capsules were used for the D-cycloserine arm of the study while two 50mg ascorbic acid capsules were used for the placebo arm.

Susan Ryan of The Guy's Hospital Pharmacy Unit (Guy's & St. Thomas' NHS Foundation Trust, London, SE1 7EH) assisted in preparing and quality-checking the D-cycloserine. Rima Gupta and Karen Kneller, clinical trials pharmacists at The National Hospital for Neurology and Neurosurgery, London assisted in dispensing the drugs to the subjects in a blinded fashion.

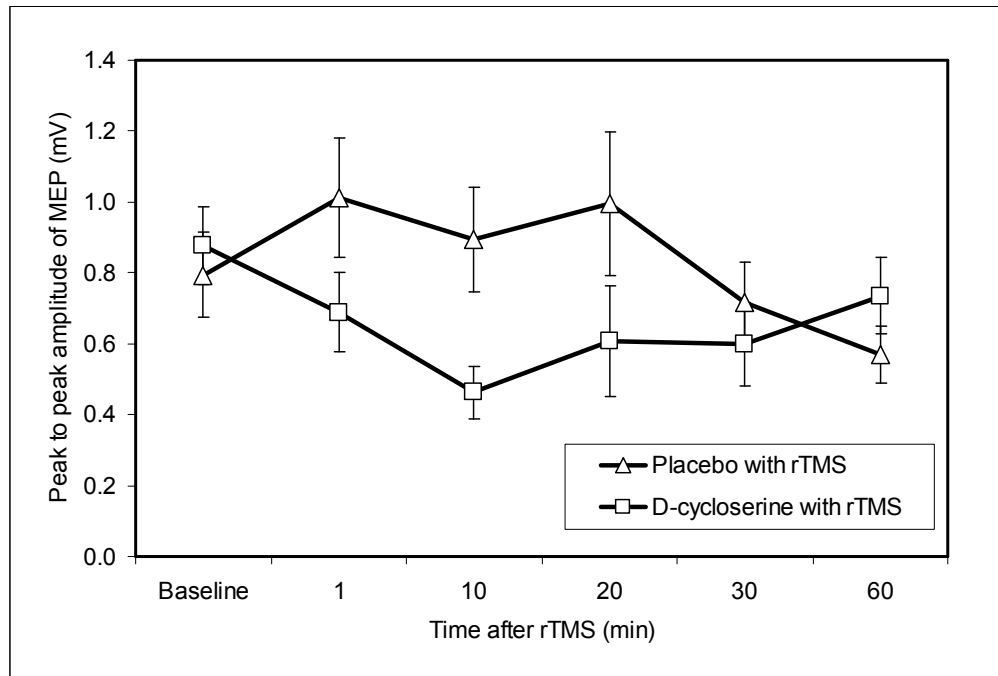


Fig 3.1: The time course of the motor-evoked potentials (MEP) after intermittent theta-burst rTMS in the placebo and D-cycloserine arm of the study. The placebo arm of the study (triangles) shows facilitation of MEPs after intermittent theta-burst rTMS while the D-cycloserine arm of the study (squares) show inhibition of MEPs after intermittent theta-burst rTMS. Error bars represent standard error of mean. Data for 6 subjects are shown.

Baseline measure	Placebo arm	D-cycloserine arm	p-value
Resting motor threshold (% of maximum stimulator output)	36.2 + 2.5	37.2 + 2.5	0.076
Active motor threshold (% of maximum stimulator output)	28.2 + 2.2	29.5 + 2.4	0.221
Intensity of test stimulation (% of maximum stimulator output)	42.8 + 2.3	43.2 + 3.2	0.890
Intensity of theta burst stimulation (% of maximum stimulator output)	33.7 + 2.1	32.8 + 2.4	0.419
MEP amplitude at baseline (mV)	0.794 + 0.12	0.877 + 0.11	0.538

Table 3.1: Comparison of baseline measures in the D-cycloserine and placebo arm of the experiment. All figures are mean + standard errors of means.

3.4 Results

No subjects suffered any adverse events from the study. Subjects could not identify whether the drug they took was D-cycloserine or placebo (40% accuracy from questionnaire recall). There was no difference in baseline RMT, AMT, MEP amplitude or intensity of test stimulation between both sessions (Table 3.1, $p > 0.05$ with student's paired t-tests for all instances).

Fig 3.1 shows the after-effects of iTBS in the D-cycloserine and the placebo arms of the study. For the placebo arm, one-factorial repeated measures ANOVA shows a significant effect of 'TIME' ($F(5,1)=3.64$, $p=0.013$). For the D-cycloserine arm of the

experiment, one-factorial repeated measures ANOVA for 'TIME' shows a significant effect of 'TIME' ($F(5,1)=2.65$, $p=0.047$). There were no post-hoc differences from baseline after Bonferroni correction in either arm of the study. In both instances, the after-effects of iTBS appear to dissipate by 60 minutes.

Two-factorial repeated measures ANOVA for 'DRUG' and 'TIME' showed a significant 'DRUG'x'TIME' interaction ($F(1,5)=4.10$, $p=0.007$) with no significant 'DRUG' effect ($F(1,5)=3.02$, $p=0.143$) or 'TIME' effect ($F(5,1)=2.22$, $p=0.139$). It is clear in Figure 3.1 that iTBS has a facilitatory after-effect on MEP amplitudes when given with placebo, but an inhibitory after-effect when given with D-cycloserine.

3.5 Discussion

This study shows that the effects of iTBS can be modulated by pharmacological intervention with an NMDA partial agonist, D-cycloserine. Moreover, in the presence of the drug the after-effects of iTBS were switched from facilitation to inhibition. It is unlikely that the D-cycloserine dosage is inappropriate for agonist action as it is identical to that used in a previous study using TDCS (Nitsche et al., 2004a). Also, it is unlikely that D-cycloserine had an effect on cortical excitability independent of iTBS, as it has been shown to have no effect on thresholds, MEPs or paired-pulse paradigms using short-interval intracortical inhibition or intracortical facilitation (Nitsche et al., 2004a).

One other possible site of action of D-cycloserine is on glycine-A strychnine-sensitive receptors that are found predominantly in the brainstem and spinal cord. However, D-cycloserine has a much lower affinity to these receptors (K_d of 90-100 μ mol/l), in

comparison to the glycine-B binding site on the NMDA receptor (K_d of 100–300nmol/l) (D'Souza et al. 1995). Previous studies of the bioavailability and pharmacokinetics of D-cycloserine show that a 150mg dose achieves 2 hour peak plasma level of 35 μ mol/l (van Berckel et al. 1997) and a 250mg dose achieves a 2 hour peak CSF level of 12.8 μ mol/l (Nair et al. 1956). Thus, at the 100mg dose used in this study D-cycloserine would not significantly bind to glycine-A receptors but would be still be sufficient to bind to the glycine-B binding site of the NMDA receptor.

On the other hand, it is not clear why the excitatory effects of TBS should be reversed into inhibition by NMDA receptor activation by D-cycloserine. In particular, this contrasts with the finding in TDCS where the excitatory effects are prolonged by D-cycloserine (Nitsche et al., 2004a). One possible explanation could lie in the suggestion (Huang et al., 2004; Huang et al., 2005) that the after-effects of TBS could be due to two simultaneous excitatory and inhibitory effects with differing time courses and strengths. It is thus conceivable that these two proposed effects have differing NMDA-dependency, and are differentially modulated by D-cycloserine. This remains to be confirmed but it is interesting to note that in the previous study with D-cycloserine the effects on TDCS were not 'symmetrical': D-cycloserine prolonged excitatory (anodal) TDCS but had no effect on inhibitory (cathodal) TDCS (Nitsche et al., 2004a).

One possible limitation of this study is the lack of measurement of D-cycloserine plasma or CSF levels. As D-cycloserine is a partial agonist, the effect is critically dependent on the bioavailability of the drug; D-cycloserine may be producing agonist

and antagonist effects on NMDA-receptors in different subjects due to the lack of control on this bioavailability. This cannot be excluded from this study, although it is unlikely the drug dose was excessive as Huang et al., 2007 showed that NMDA-antagonism completely blocks theta burst rTMS rather than altering its after-effects.

In rat sensorimotor cortex, the effects of theta burst stimulation is dependent on NMDA receptors and the effects can be modulated by calcium channel blockade (Castro-Alamancos et al., 1995) and this effect can be reversed depending on extracellular calcium concentration (Barr et al., 1995). This reversal of the effect of theta burst stimulation has also been observed with theta burst rTMS in humans with reversal of effects depending on pre-existing cortical state (Gentner et al., 2008) and immediate activity (Huang et al., 2008). Thus, it is conceivable that D-cycloserine may have a similar effect on theta burst rTMS in humans by converting LTP induced by iTBS into impaired excitability (i.e. LTD).

The next step would be to try and modulate the effects of TBS using drugs which act on known neuromodulators like acetylcholine, dopamine and noradrenaline. This is the focus of the next chapter.

Chapter 4

Theta burst stimulation and neuromodulatory drugs

Work described in this chapter is being prepared submission for publication:

Swayne OB, Teo JT, Cheeran BJ, Greenwood R, Rothwell JC. The interaction between human theta burst stimulation and nicotine reveals a role for performance variability in motor learning (preparing for submission to Cerebral Cortex).

4.1 Introduction

The previous chapter demonstrated that the effect of theta burst stimulation is modulated by activity at the NMDA receptor, the initial molecular trigger of long term potentiation as studied in animals.

Long term potentiation is also known to be modulated by a variety of other neurotransmitters: the noradrenergic system, the dopaminergic system, the cholinergic system and the GABA-ergic system have all been shown to alter the duration, degree and persistence of long term potentiation (see section 1.3 in the Introduction). The mechanism of action varies depending on the neurotransmitter, as different receptors intervene at different points of the molecular pathway:

As mentioned in Chapter 1, dopaminergic receptors have been suggested to play a role in modulating striatal and hippocampal plasticity, as well as in homeostatic plasticity and metaplasticity. Likewise nicotinic acetylcholine receptors and adrenergic receptors have also been implicated in animal models of plasticity as well.

Thus, based on the findings in the literature of animal experiments with theta burst stimulation, this chapter will explore the effects of various neurotransmitter systems on the after-effects of theta burst stimulation: study A explored the noradrenergic and dopaminergic system using amphetamine and levo-dopa and study B used nicotine to study the cholinergic system.

4.2 Amphetamine & levodopa

In humans, a comprehensive study of cholinergic, noradrenergic and dopaminergic systems in practice-dependent plasticity showed that noradrenergic activation enhances this plasticity transiently while cholinergic and dopaminergic activation enhances practice-dependent plasticity beyond the period of training (Meintschel et al., 2006).

This strongly suggests a role for dopaminergic and cholinergic systems in human motor system plasticity. The role of dopamine receptors in modulating LTP in the striatum is well-established in animal models (Centonze et al., 1999; Calebresi et al., 2007), so it is reasonable to expect that activation of dopaminergic system would enhance rTMS-induced plasticity if the latter is analogous to LTP.

Activation of noradrenergic and dopaminergic systems also affect cortical excitability independent of plasticity changes. Amphetamine has been shown to enhance MEP amplitude (Boroojerdi et al., 2001a; Ziemann, 2004). Thus any study of these two neuromodulatory systems will have to consider the drug effects on cortical excitability independent of rTMS-induced plastic changes.

In this study, we aimed to see if rTMS-induced plasticity can be enhanced in the same way as practice-dependent plasticity was by Meintszchel et al. (2006) using the drugs, dexamphetamine and L-DOPA in healthy normal subjects.

4.2.1 Study design

A double-blind randomised placebo-controlled crossover trial was conducted. 10 subjects (4 females, 6 males; 31.1 ± 5.0 SD years) were recruited for this study. Subjects received amphetamine, L-DOPA or placebo before the delivery of rTMS, which was timed to coincide with the peak plasma concentration of the drug (2.5 hour for amphetamine, 1 hour for L-DOPA and 2 hour for placebo). After rTMS, MEPs were then recorded immediately and 5, 10, 20, 30 and 40 minutes after rTMS. Paired-pulse stimulation with an interstimulus interval of 2ms measuring short-interval intracortical inhibition (SICI) was also recorded every 5 minutes up to 20 minutes, then less frequently up to 40 minutes.

In all cases, MEPs were recorded from the left FDI (TMS pulses on the right primary motor cortex). The rTMS protocol used was intermittent theta-burst stimulation (iTBS) which is an excitatory protocol (Huang et al. 2005).

4.2.1.1 Drug

Two Madopar 100/25 capsules (Roche Products Ltd) and two Dexamphetamine 5mg tablets (Celltech, UCB Pharma Ltd.) were used in the L-DOPA and the dexamphetamine arms of the study respectively. Two 50mg ascorbic acid mint-flavoured lozenges were used for the placebo arm of the experiment. Both investigator and subjects were blinded to the drug.

4.2.1.2 Statistical analysis

A two-factorial analysis of variance (ANOVA) with repeated measures were used with factors: “DRUG” (Amphetamine, L-DOPA, Placebo) and “TIME” (Baseline, 0min, 10min, 20min, 30min, 40min) for only the 6 subjects who completed all three drug arms (n=6).

Additionally, as 10 subjects completed the amphetamine and placebo arms, two non-independent ANOVAs were also performed comparing only the amphetamine or L-DOPA with the placebo arm. Any significance is corrected by a Bonferroni correction due to the two non-independent comparisons.

4.2.2 Results

No subjects suffered any adverse events from the study. Subjects were able to identify accurately when amphetamine was taken (70% accuracy) due to a subjective feeling of euphoria. However, subjects did not experience any significant side effects with either

L-DOPA or placebo but was able to identify above chance which drug was taken (L-DOPA at 67% accuracy, placebo at 60% accuracy).

There was no difference in baseline RMT, AMT, MEP amplitude or intensity of test stimulation between both sessions ($p > 0.05$ with student's paired t-tests for all instances). This is summarised in Table 4.1.

Baseline measure	Placebo arm	Amphetamine arm	L-DOPA arm
Intensity of test stimulation (% of maximum stimulator output)	47.5 ± 3.9	45.2 ± 3.6	44.8 ± 3.1
Intensity of paired-pulse stimulation (% of maximum stimulator output)	24.5 ± 1.60	22.7 ± 0.97	24.0 ± 1.73
Intensity of theta burst stimulation (% of maximum stimulator output)	36.4 ± 2.4	36.6 ± 2.4	34.8 ± 2.7
MEP amplitude at baseline (mV)	1.08 ± 0.11	1.07 ± 0.13	1.19 ± 0.14

Table 4.1: Comparison of baseline measures in the amphetamine, L-DOPA and placebo arm of the experiment. All figures are mean \pm standard errors of means.

Fig 4.1 shows the after-effects of intermittent theta-burst stimulation in the amphetamine and the placebo arms of the study for 10 subjects, and Fig 4.2 shows the after-effects of iTBS in the L-DOPA and placebo arms of the study for 6 subjects. The three factorial ANOVA with all three drug arms and only the 6 subjects which completed the study

When directly comparing only the amphetamine and placebo arm of the experiment with 10 subjects, there were no significant differences between both arms of the experiment as analysed by a two-factorial ANOVA for “DRUG” or “TIME” ($p>0.05$ for DRUG effect, TIME effect and DRUG x TIME effect. When directly comparing only the L-DOPA and placebo arm of the experiment with 6 subjects, there were no significant differences between both arms of the experiment as analysed by a two-factorial ANOVA for “DRUG” or “TIME” ($p>0.05$ for DRUG effect, TIME effect and DRUG x TIME effect). There was also no significant effects on SICI as measured by paired-pulse stimulation (data not shown, $p>0.05$).

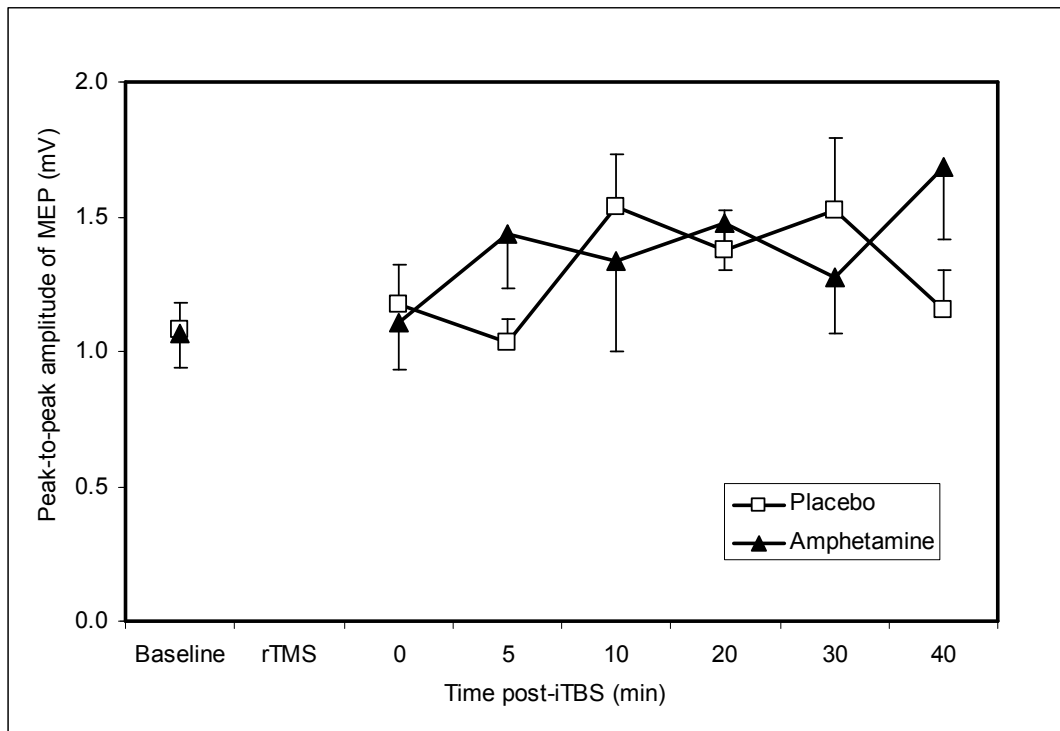


Fig 4.1: The time course of the motor-evoked potentials (MEP) after intermittent theta-burst rTMS in the placebo and amphetamine arm of the study. The placebo arm of the study (squares) shows facilitation of MEPs after intermittent theta-burst rTMS while the amphetamine arm of the study (filled triangles) also showed facilitation of MEPs after intermittent

theta-burst rTMS. Errors bars represent standard error of mean. Data for 10 subjects are shown.

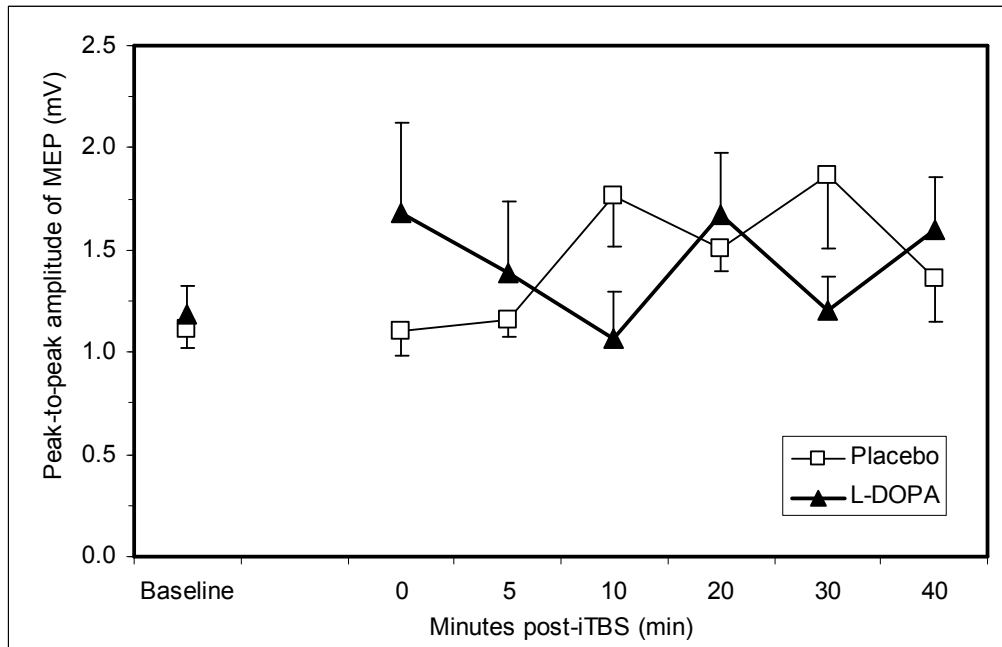


Fig 4.2: The time course of the motor-evoked potentials (MEP) after intermittent theta-burst rTMS in the placebo and L-DOPA arm of the study. The placebo arm of the study (squares) shows facilitation of MEPs after intermittent theta-burst rTMS while the L-DOPA arm of the study (filled triangles) show inhibition of MEPs after intermittent theta-burst rTMS. Errors bars represent standard error of mean. Data for 6 subjects are shown.

The lack of effect of either drug compared to placebo when combined with intermittent theta-burst stimulation resulted in termination of this study so no control experiments with either drug without stimulation was conducted.

4.2.3 Discussion

There is a lack of effect of amphetamine and L-DOPA compared to placebo on the after-effects of TBS. There is no good explanation for this since these two drugs are known to modulate the after-effects of transcranial direct current stimulation (Nitsche et al., 2004b; Nitsche et al., 2006) and practice-dependent plasticity (Tegenthoff et al., 2004; Meintzchel et al., 2006). Additionally amphetamine has been shown to modulate rTMS-induced plasticity in a complex fashion: it suppresses MEP enhancement from rTMS during ischaemic nerve block despite enhancing MEP during ischaemic nerve block alone (Ziemann et al., 2002). Thus the lack of effect in study B could be related to the paradigm used (i.e. theta burst stimulation) which may be similar but not identical to the paradigms used in previous studies.

However, it should be noted there is a very strong effect of TBS in the placebo arm of the experiment (Fig 4.1 and Fig 4.2). This strong effect is much more potent than the effect seen in the original description of TBS (Huang et al., 2005), lasting in excess of 20 minutes. Thus one possible explanation is that the placebo effects are particularly potent. Certainly some subjects described the expectation of possibly receiving amphetamine as 'exciting'. Thus, it is conceivable that this associated anticipation may have obscured the results of amphetamine since emotional states are known to change in neurotransmitter levels and cortical excitability as measured by MEPs.

The lack of effect with L-DOPA is also disappointing. L-DOPA has been shown to affect practice-dependent plasticity most prominently in the elderly as well as in chronic stroke patients (Floel et al., 2005a & 2005b). The likely explanation for the absence of effect is probably due to an error in study design: young subjects (24-35 years of age) were used in this study (unlike the above studies) and one would expect

that there might be a ‘ceiling’ effect in young normal subjects who are not deficient in dopaminergic transmission.

Thus, no firm conclusions can be drawn from study B about the role of noradrenergic and dopaminergic systems in rTMS-induced plasticity in particular theta-burst-induced plasticity. The experimental design will need closer scrutiny in the next experiment, where the role of nicotinic acetylcholine receptors in rTMS-induced plasticity is studied.

4.3 Nicotine

Acetylcholine receptors are widely distributed in the central nervous system and are divided into nicotinic acetylcholine receptors and muscarinic acetylcholine receptors. Both receptor types are expressed in sensorimotor cortex with variable expression on different interneuron populations (Sihver et al., 1998; Alkondon et al., 2000). There is significant evidence for muscarinic acetylcholine receptors playing a role in the cortico-cortical circuit mediating short afferent inhibition (SAI), as the muscarinic antagonist scopolamine inhibits SAI (Di Lazzaro et al., 2000) and SAI has been demonstrated to be deficient in Alzheimer’s disease but not in fronto-temporal dementia (Di Lazzaro et al., 2004) or mild cognitive impairment (Sakuma et al., 2007). The acetylcholinesterase inhibitor, taurine, has also been shown to affect intracortical circuits by decreasing short-interval intracortical inhibition, SICI (Korchounov et al., 2005).

Nicotinic acetylcholine receptors are known to be expressed on interneurons in the cerebral cortex including the somatosensory and primary motor cortex (Sihver et al., 1998; Alkondon et al., 2000; Xiang et al., 1998; Christophe et al., 2002). In humans, nicotine while not altering SAI, SICI or MEPs in normal subjects, corrects the abnormalities in SICI seen in patients with Tourette’s syndrome (Orth et al. 2005). This

suggests that nicotinic acetylcholine receptors have a role to play in modulating motor cortex activity despite not being easily detectable in normal subjects using single or paired-pulse TMS.

Nicotinic acetylcholine receptors are also known to be involved in various animal models of hippocampal and striatal synaptic plasticity. Nicotine enhances presynaptic and postsynaptic LTP: nicotine can also enhance the generation of LTP (Ge & Dani, 2005), via presynaptic effects on glutamate release in the ventral tegmentum (Mansvelder & McGehee, 2000), and nicotine enhances LTP induction primarily through activation of postsynaptic nAChRs in the hippocampus (Fujii et al., 1999; Ji et al., 2001). Thus, the purpose of this study is to determine if activating nicotinic acetylcholine receptors with nicotine can modulate rTMS-induced plasticity in the primary motor cortex in normal healthy non-smoking subjects.

4.3.1 Study design

A double-blind randomised placebo-controlled crossover trial was conducted with subjects receiving either nicotine or placebo 1 hour before the delivery of rTMS. 10 subjects (3 females, 7 males; 29.6 ± 4.7 SD years) were recruited.

After rTMS, MEPs were then recorded every 5 minutes for 40 minutes. Paired-pulse stimulation with an interstimulus interval of 2ms measuring SICI was also recorded every 5 minutes up to 20 minutes, then less frequently up to 40 minutes. Additionally, in a separate control experiment investigating the effects of nicotine independent of rTMS, subjects received nicotine and had MEPs measured for 40 minutes. Current smokers and recent ex-smokers, within the past year, were excluded from the study.

In all cases, MEPs were recorded from the left FDI (TMS pulses on the right primary motor cortex). The rTMS protocol used was intermittent theta-burst stimulation (iTBS) which is an excitatory protocol (Huang et al. 2005) lasting about 20 minutes.

4.3.1.1 Drug

Two 2mg mint-flavoured nicotine lozenges were used for the nicotine arm of the experiment. Lozenges were selected because of the fast pharmacokinetics profile with rapid absorption (Hukkanen et al., 2005; Russel, 1987; Tobacco Advisory Group, 2000); additionally, swallowing the tablet does not significantly alter the pharmacokinetics profile of the nicotine (Choi et al., 2003). Two 50mg ascorbic acid mint-flavoured lozenges were used for the placebo arm of the experiment. To mask the distinctive taste of the nicotine lozenges subjects also took strong menthol lozenges (Fisherman's Friend) before taking either drug and continued to take the menthol lozenges while they had the drug in the mouth. Both investigator and subjects were blinded to the drug taken during each session.

4.3.1.2 Statistical analysis

Each drug arm was compared individually with the placebo arm in their respective studies using a two-factorial analysis of variance (ANOVA) with repeated measures were used with factors: "DRUG" and "TIME".

Experimental arms compared	Factor 1	Factor 2
Nicotine vs. placebo	"DRUG"	"TIME": Baseline, 0-5min, 10-15min, 20-25min, 30-35min, 40min
Nicotine-alone vs. Nicotine-rTMS	"rTMS"	"TIME": Baseline, 0-5min, 10-15min, 20-25min, 30-35min, 40min

4.3.2 Results

None of the subjects reported any adverse events from the rTMS. Two subjects reported a sensation of nausea associated with taking the nicotine, but were happy to proceed. Due to the associated nausea, the blinding of the experiment was likely to be compromised as the 80% of the placebo group and 80% of the nicotine group could accurately guess the drug taken after each session. There were no significant differences at baseline for RMT, AMT, intensity of test stimulation and intensity of theta-burst stimulation between all arms of the experiment (Table 4.2, $p > 0.05$). MEP measurements at some time points were skewed did not have a normal distribution, so log10 transformation was performed.

Baseline measure	Placebo-rTMS arm	Nicotine-rTMS arm	p-value
Active motor threshold (% of maximum stimulator output)	47.1 ± 2.9	48.3 ± 1.9	0.519
Intensity of test stimulation (% of maximum stimulator output)	58.1 ± 3.7	55.4 ± 3.3	0.372
Intensity of theta burst stimulation (% of maximum stimulator output)	37.7 ± 2.3	38.6 ± 1.5	0.519
MEP amplitude at baseline (mV)	0.920 ± 0.139	1.111 ± 0.217	0.433

Table 4.2: Comparison of baseline measures in the placebo-rTMS arm and nicotine-rTMS arm of the experiment. All figures are mean \pm standard errors of means.

In the placebo arm, corticospinal excitability as measured by MEPs rose after the rTMS but returned to normal within 10 minutes, while in the nicotine arm, corticospinal

excitability rose slowly after rTMS significantly more than the placebo arm and lasted more than 20 minutes (Fig 4.3).

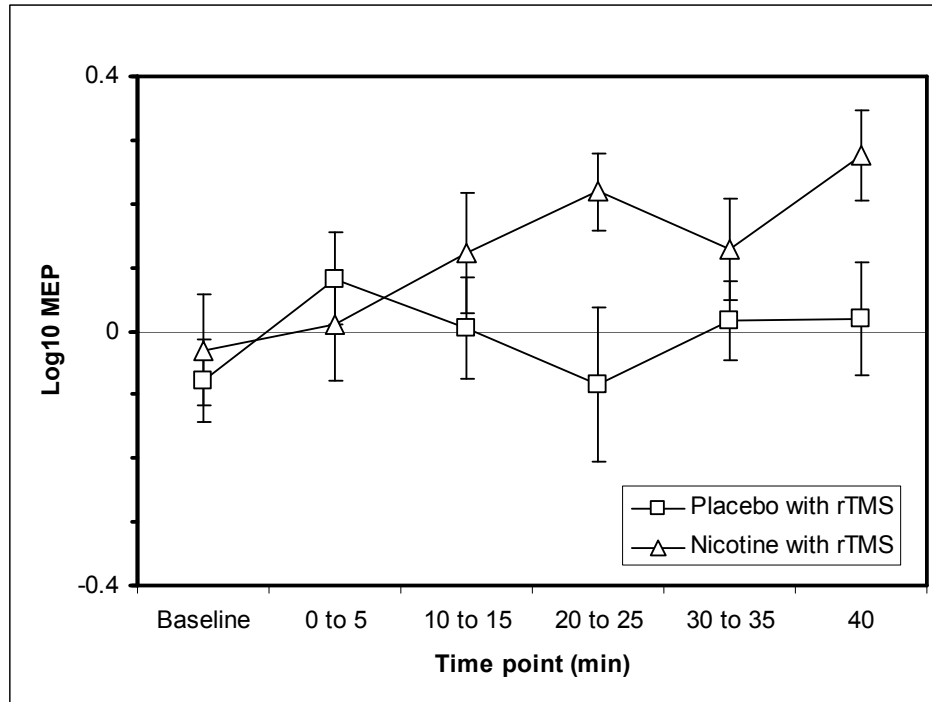


Fig 4.3: The time course of the motor-evoked potentials (MEP) after intermittent theta-burst stimulation in the placebo and nicotine arm of the study. The placebo arm of the study (squares) shows initial facilitation of MEPs after iTBS while the nicotine arm of the study (triangle) show further enhancement of MEPs after iTBS. Data for 10 subjects are shown.

Two factorial ANOVA with repeated-measures using the factors “DRUG” and “TIME” showed a significant interaction of “DRUG” X “TIME” ($F=4.32$, $p=0.02$) with no significant effect of “DRUG” ($F=2.089$, $p=0.182$) or “TIME” ($F=2.917$, $p=0.054$) independently. Post-hoc tests showing a facilitatory effect in the nicotine arm of the experiment at 10-15 minutes, 20-25 minutes, 30-35 minutes and 40 minutes ($p=0.014$,

$p=0.006$, $p=0.002$ and $p=0.002$ respectively). On Fig 4.3, it is clear that the nicotine arm has a larger and longer-lasting MEP enhancement after iTBS compared with the placebo arm.

As a control for the effect of nicotine on MEPs in the absence of intermittent TBS, a control experiment was conducted where subjects took nicotine without receiving any intermittent TBS. Only 9 out of the original 10 subjects were able to participate in this control experiment. MEPs were not changed by nicotine alone (Fig 4.4) compared with nicotine with rTMS showed an interaction effect of “rTMS” and “TIME” as well ($F=4.660$; $p=0.012$) and a significant independent effect of “rTMS” ($F=6.77$, $p=0.032$) but not “TIME” ($F=1.11$, $p=0.372$). Thus MEP after-effects of intermittent TBS interacted with the nicotine despite nicotine not having any significant effect on MEPs alone.

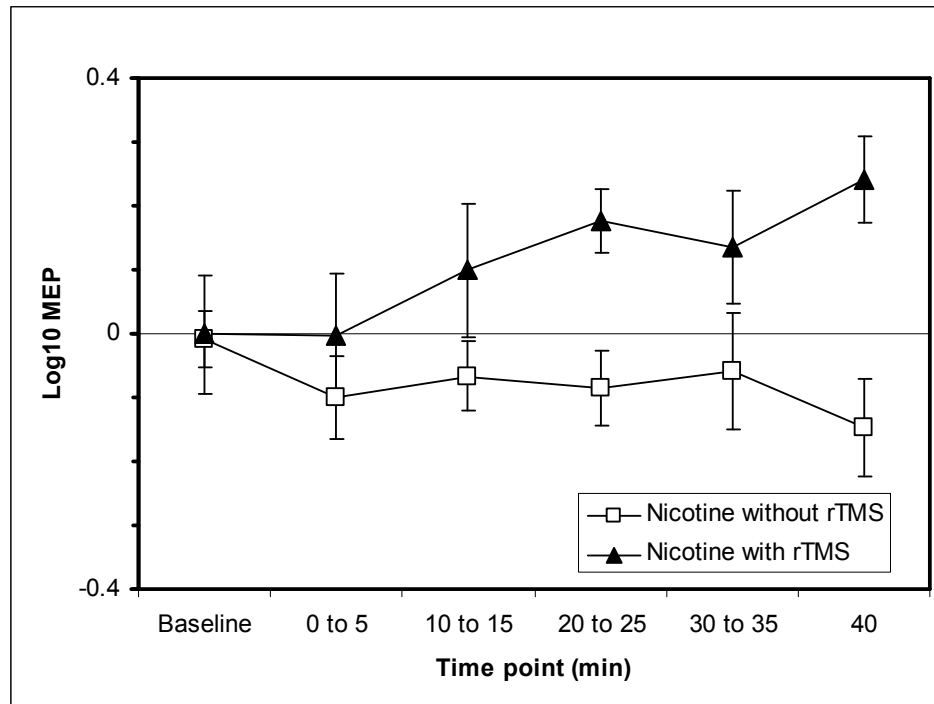


Fig 4.4: The time course of the motor-evoked potentials (MEP) after ingestion of nicotine with or without intermittent theta burst stimulation.

The rTMS arm of the study (filled triangles) shows facilitation of MEPs after iTBS and nicotine while the nicotine without rTMS arm of the study (squares) does not have any significant effect on MEPs. Data for 9 subjects are shown (1 subject dropped out from one arm).

Paired pulse stimulation of corticospinal excitability to measure SICI did not show any significant differences before and after rTMS in either experimental arm (data not shown, $p > 0.05$).

4.3.3 Discussion

The transient increase in MEP amplitudes following iTBS delivered to the motor cortex was first described in 2005 (Huang et al 2005) and has been widely reproduced (Di Lazzaro et al 2006; Huang et al 2007a; Huang et al 2007b). With nicotine this increase was more marked, started later and was more prolonged. Excitability was still increased at 40 minutes, and it is unclear how long this would have persisted. Nicotine alone did not alter excitability, consistent with previous reports (Orth et al 2005), suggesting that the effect resulted from an iTBS-drug interaction. As nicotine up-regulates LTP this would seem a reasonable candidate mechanism for enhancing the effect of iTBS on corticospinal excitability.

Thus nicotine has been identified as an agent that can facilitate and prolong the enhancement of corticospinal excitability by intermittent theta-burst stimulation. It is therefore hypothesised that using nicotine to enhance the effect of intermittent theta-burst stimulation on corticospinal excitability might also enhance motor learning. If this was the case, nicotine and intermittent theta burst stimulation may be an attractive tool for clinical application in rehabilitation and learning. This is the focus of the next chapter that studied the effect of nicotine, TBS and motor learning.

Chapter 5

Theta burst stimulation and ballistic motor learning

Work described in this chapter has been submitted to:

Swayne OB, Teo JT, Greenwood R, Rothwell JC. Nicotine modulates the effects of theta burst stimulation (accepted by Clinical Neurophysiology; 22nd June 2009).

Teo JT, Swayne OB, Cheeran BJ, Greenwood R, Rothwell JC. Human theta burst stimulation enhances subsequent motor learning while increasing performance variability (submitted to Cerebral Cortex; 27th June 2009).

5.1 Introduction

From Chapter 4, nicotine has been identified as an agent that can facilitate and prolong the enhancement of corticospinal excitability of intermittent theta-burst stimulation. It is therefore hypothesised that using nicotine to enhance the effect of intermittent theta-burst stimulation on corticospinal excitability might also enhance motor learning. If this was the case, nicotine and intermittent theta burst stimulation may be an attractive tool for clinical application in rehabilitation and learning. A simple ballistic motor task was used to assess motor learning as it is well-established to be dependent in its early phase on the primary motor cortex (Muellbacher et al., 2001; Muellbacher et al., 2002). This makes the ballistic motor learning paradigm more suitable for study compared to other paradigms of motor learning which have not been shown to involve the primary motor cortex, like force field adaptation learning (Baraduc et al., 2004) or serial reaction time tasks which involve learning of abstract sequences which may or may not be dependent on the primary motor cortex.

5.2 Study Design

In this study, the same nicotine and rTMS protocol as in study C (see Chapter 4) was reproduced, and the subject was required to perform a motor learning task during the period of peak MEP enhancement according to study C (10-20 minutes). A new group of 10 subjects (2 females, 8 males; 29.5 ± 4.1 SD years) were recruited for this study. MEP measurements were not made during the motor learning as any measurements would not be reliable during activity. This study was conducted as a randomised controlled blinded trial with a cross-over design over the four experimental arms (placebo and sham-rTMS, placebo and real-rTMS, nicotine and sham-rTMS, and

nicotine and real-rTMS). The order of the sessions was pseudo-randomised. To minimise carry-over effects in a motor learning task, intersession gap was kept very long (mean 31 days, range 14-55 days). Subject and investigator were both blinded to the drug, and subjects were also blinded to whether real-rTMS or sham-rTMS was delivered. Current smokers and recent ex-smokers (within the past year) were excluded from the study.

5.2.1 Ballistic motor learning

Motor learning was assessed using a simple ballistic motor task as previously described (Muellbacher et al., 2001; Muellbacher et al., 2002). This task was chosen as it subjectively resembles the efforts made by stroke patients when trying to move a paretic limb with more force, as opposed to other motor learning tasks which often do not resemble motor learning as it occurs in a rehabilitative setting.

The left hand was positioned supine on a board with the wrist, metacarpophalangeal and distal interphalangeal joints fixed with Velcro straps. The thumb was left unsecured and could abduct and oppose freely. A piezoresistive monoaxial accelerometer (Model SA-105 vibrometer, Fribourg, Switzerland) was attached on the lateral aspect of the left thumb proximal phalanx with the maximal vector being thumb abduction. The accelerometer signal was sampled at 5000Hz and not filtered. The left (non-dominant) thumb was used in all conditions to minimise ceiling effects (which may occur in the dominant hand).

Subjects were familiarized with the motor task (to abduct the left thumb as quickly as possible in the direction of the accelerometer in time with a 0.5Hz audio metronome to maximize initial peak acceleration using the computer monitor for explicit visual feedback. The monitor displayed the last three thumb abductions. The investigator

motivated the subject by providing a target ~10% above the highest acceleration in the last three thumb abductions. Subjects were allowed ten successful movements for familiarization before each experiment. Successful movements were defined as rapid ballistic thumb abduction movements of at least 1g during the first 100ms of movement. Subjects were instructed to maintain the original thumb position by ensuring that the accelerometer signal returned to baseline ($\pm 0.05g$) after each movement. Subjects performed 6 training blocks separated by rest blocks of 1 minute. Each training block lasted 1 minute, consisting of 30 training movements. Unsuccessful training movements were not compensated for unless < 27 successful training movements ($< 90\%$) were performed in the training block.

5.2.2 Data analysis

For study D, to exclude baseline differences, data from Block1 of each experimental arm was also compared in a two-factorial ANOVA with repeated measured using factors “DRUG” (Nicotine or placebo) and “rTMS” (Sham-rTMS or real-rTMS). Three factorial ANOVA with repeated-measures was then conducted using the factors “DRUG”, “rTMS” and “BLOCK”. If there was significant interaction of “DRUG”, “rTMS” and “BLOCK”, two factorial ANOVAs were done comparing the learning for all four arms of the experiment as follows:

Arm	v	Arm	Factor 1	Factor 2
Sham-rTMS Placebo	v	Sham- rTMS Nicotine	“DRUG” (Nicotine, placebo)	“BLOCK” (Block1 to Block6)
Sham-rTMS Placebo	v	Real-rTMS Placebo	“rTMS” (Real-rTMS, sham-rTMS)	“BLOCK” (Block1 to Block6)
Sham-rTMS Placebo	v	Real-rTMS Nicotine	“2x INTEVENTION” (Active, sham)	“BLOCK” (Block1 to Block6)
Real-rTMS Placebo	v	Real-rTMS Nicotine	“DRUG” (Nicotine, placebo)	“BLOCK” (Block1 to Block6)
Sham-rTMS Nicotine	v	Real-rTMS Nicotine	“rTMS” (Real-rTMS, sham-rTMS)	“BLOCK” (Block1 to Block6)
Sham-rTMS Nicotine	v	Real-rTMS Placebo	“INTERVENTION” (Nicotine, real-rTMS)	“BLOCK” (Block1 to Block6)

5.3 Results

None of the subjects reported any adverse events to the drug or the rTMS. In all arms of the experiment, subjects improved in their initial peak acceleration over 6 blocks (Fig 5.1). Firstly, to confirm that learning occurs in this paradigm as previously reported, a one-way ANOVA with repeated-measures of “BLOCK” on the sham-rTMS and placebo arm of the experiment was performed and showed a significant effect of “BLOCK” ($F=4.05$, $p=0.025$). Initial peak acceleration improved from mean of 1.49g to 1.82g (~20%) after 6 blocks of practice (6 minutes of practice or 180 movements) (Fig 5.1). This results reproduce the results of previous studies of motor learning (Muellbacher et al., 2001; Muellbacher et al., 2002; Baraduc et al., 2004) and is also comparable with previous studies where 30 minutes of practice (or 900 movements) produced about 60% increase in performance.

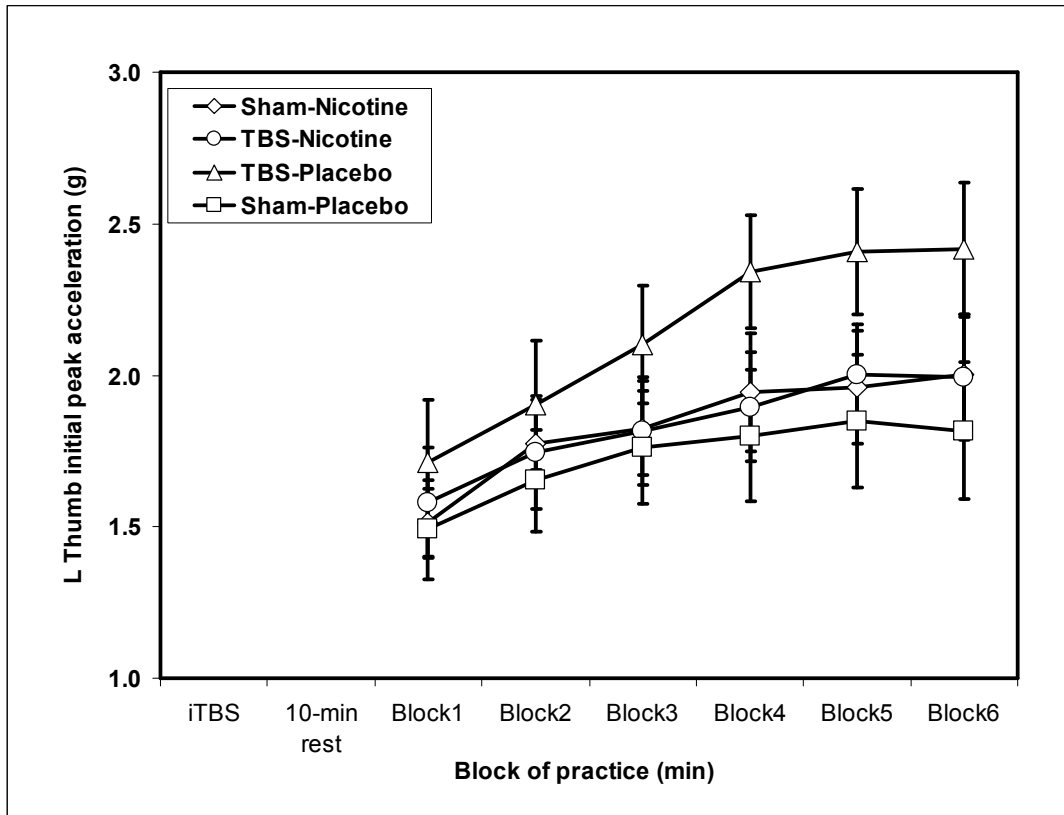


Fig 5.1: Peak initial acceleration of left thumb abduction after drug and/or stimulation over 6 blocks of 0.5Hz ballistic thumb training. Each block is separated by 1 min of rest. Four experimental arms are represented: sham stimulation with placebo (square), sham stimulation with nicotine (diamond), theta-burst stimulation with placebo (triangles) and theta-burst stimulation with nicotine (circle). Data for 10 subjects are shown.

For analysis of all four experimental arms, a three-factorial ANOVA with repeated-measures using the factors “DRUG”, “rTMS” and “BLOCK” showed a significant interaction of “DRUG” x “rTMS” x “BLOCK” ($F=2.70$, $p=0.032$) (Table 5.1). It is apparent on the Fig 5.1 that three arms of the experiment have similar rates of motor learning: sham-rTMS and placebo, sham-rTMS and nicotine, and real-rTMS and nicotine, while the real-rTMS and placebo had a higher rate of motor learning. This is confirmed with two-factorial ANOVA comparing real-rTMS and placebo with sham-rTMS and placebo which showed a significant interaction of “rTMS” x “BLOCK” ($F=3.21$, $p=0.049$) and a two-factorial ANOVA comparing real-rTMS and nicotine “DRUG” x “BLOCK” ($F=2.73$, $p=0.031$). Finally, sham-TBS with nicotine did not have any significant effect on the rate of motor learning ($F=0.50$, $p=0.648$). The results of the statistical analysis are summarised in Table 5.1.

The intersession gap between subjects were kept very long (mean = 31 days, range 14-55 days) to minimise carry-over effects. Baseline measures at the beginning of each learning session (Block1) showed no significant interaction of “DRUG”, “rTMS” or “DRUG” x “rTMS” indicating that there was no significant carryover effect detected or any significant effect of either intervention (rTMS or drug) on baseline motor performance (Table 5.2).

Table A: Three-factorial ANOVA with repeated-measures of all experimental arms		
Factor(s)	F	p
DRUG	1.07	0.328
rTMS	3.81	0.083
BLOCK	35.2	<0.001***
DRUG x rTMS	4.70	0.058
DRUG x BLOCK	0.762	0.506
rTMS x BLOCK	1.31	0.294
DRUG x rTMS x BLOCK	2.70	0.032*

Table B: Two-factorial ANOVA with repeated measures comparing individual experimental arms			
Arms compared	Factor(s)	F	p
Sham-rTMS and Placebo vs. Sham-rTMS and Nicotine	DRUG	1.15	0.311
	BLOCK	7.62	0.003**
	DRUG x BLOCK	0.50	0.648
Sham-rTMS and Placebo vs. Real-rTMS and Placebo	rTMS	5.36	0.046*
	BLOCK	20.8	<0.001***
	rTMS x BLOCK	3.21	0.049*
Sham-rTMS and Placebo vs. Real-rTMS and Nicotine	INTERVENTION	0.30	0.297
	BLOCK	13.6	<0.001***
	INTERVENTION x BLOCK	0.319	0.747

Real-rTMS and Placebo vs. Real-rTMS and Nicotine	DRUG	0.001	0.975
	BLOCK	16.3	<0.001***
	DRUG x BLOCK	0.260	0.933
Sham-rTMS and Nicotine vs. Real-rTMS and Nicotine	rTMS	3.52	0.094
	BLOCK	37.2	<0.001***
	rTMS x BLOCK	2.73	0.031*
Sham-rTMS and Nicotine vs. Real-rTMS and Placebo	INTERVENTION	3.02	0.116
	BLOCK	27.0	<0.001***
	INTERVENTION x BLOCK	1.54	0.235

Table 5.1: Analysis of Variance (ANOVA) of ballistic motor learning.

(A) Three-factorial ANOVA with repeated measures of “DRUG” x “rTMS” x “BLOCK”; (B) Two-factorial ANOVA with repeated measures comparing the four different arms of the experiment with each other. * indicates $p < 0.05$, ** indicates $p < 0.01$ and *** indicates $p < 0.001$.

Table 6: Two-factorial ANOVA with repeated-measures of baseline measures (Block1 only)		
Factor(s)	F	p
DRUG	0.300	0.597
rTMS	0.885	0.371
DRUG x rTMS	0.607	0.456

Table 5.2: Analysis of Variance (ANOVA) of baseline block1 of all experimental arms showing no significant carryover effect at baseline or effect of the intervention on baseline motor performance.

5.4 Discussion in the interlude

5.4.1 The effect of iTBS on motor learning

iTBS increased the rate at which subjects improved performance of the thumb abduction task without affecting baseline measures. To our knowledge, this is the first report in healthy volunteers in which excitatory rTMS enhances subsequent motor learning. A recent study found that training in a finger abduction task was not affected by 5 Hz rTMS (Agostino et al 2007) or iTBS (Agostino et al., 2008). However, this study involved motor performance assessments both before and after rTMS, with likely consequent carry-over effects. Additionally, performance feedback of peak acceleration was not provided which is central to enhancing performance. In a group of stroke patients 10 Hz rTMS enhanced the acquisition of a serial reaction time task but unlike the present study stimulation was interspersed with task practice, making it difficult to distinguish improved performance from improved learning (Kim et al 2006).

Improvement in initial peak acceleration involves two processes. First, there must be a driver to change, such that performance on a trial differs from, and on average is better than, that of the previous trial. Second, any beneficial changes in output should be stabilised, perhaps by changes in synaptic connectivity. Indeed, motor learning is accompanied by LTP within M1 (Rioult-Pedotti et al 2000) that likely involves synaptic strengthening in selected pathways. iTBS is thought to act at a cortical level (Di Lazzaro et al 2005), and can promote changes in synaptic strength (Huang et al 2005, 2007) that are thought to involve LTP (Huang et al 2007; Teo et al 2007): it thus seems a possibility that enhanced learning following iTBS may occur via increased synaptic activity with up-regulation of LTP.

5.4.2 The effect of the iTBS-nicotine interaction on learning

Nicotine alone did not affect training, but when combined with iTBS it blocked the positive effects observed in the placebo arm. This is arguably a surprising result.

Nicotinic receptors are expressed widely throughout the brain, including within M1 (Sihver et al 1998; Alkondon et al 2000), and cholinergic modulation of plasticity protocols has been demonstrated (Kuo et al 2007). Furthermore, pre- or post-synaptic enhancement of LTP by nicotine is well documented in animal models (Fisher et al 1998; Mansvelder & McGehee 2000; Ji et al 2001; Ge & Dani 2005). One may therefore have expected nicotine to enhance task acquisition.

Experiments of iTBS on motor learning above and on MEP (Chapter 4) showed that iTBS and nicotine interacted differently with regard to motor learning and corticospinal excitability. This apparent dissociation could potentially be explained by an unpredictable action of nicotine at receptors outside the motor cortex, for example in the striatum or the cerebellum (Paterson & Nordberg 2000; Gotti & Clementi 2004). In this case, however, one would expect nicotine alone to modulate motor learning, which was not the case.

One explanation is the BCM model where higher levels of excitability occlude or impair further learning (but one would expect equivalent levels of learning to TBS alone). Also others have suggested that this type of sliding threshold plays a limited role in motor learning (Siebner et al., 2004; Kuo et al., 2008) (Note: these references refer to the BCM rule as homeostatic plasticity which is a confusion of nomenclature compared to molecular models of homeostatic plasticity).

An alternative possibility is that the improvement in performance is not driven by corticospinal excitability. A presumption of the original hypothesis is that enhanced corticospinal excitability would drive more performance improvement under Hebbian principles as synaptic efficacy is enhanced and thus there would be enhanced encoding of motor memory or a learning rule. We tested if this presumption is correct by analysing the behavioural data for evidence of acquisition of a learning rule.

5.4.3 Trial-by-trial analysis of data

Trial-by-trial data of each individual subject session was analysed. Within a block, the probability of an individual trial would be better than the previous trial was calculated with a simple formula:

$$P = t(x) / T$$

where P is the probability of an individual trial being better than the previous trial; t(x) is the number of trials where performance in xth trial is greater than (x-1)th trial; and T is the total number of trials in the block.

Thus the hypothesis is that if an underlying strategy, learning rule or motor memory was being acquired, improving performance would be linked to increasing P across blocks and if TBS was enhancing learning through enhancing corticospinal excitability, sessions with active iTBS intervention would have a higher P than sessions with a sham-TBS intervention.

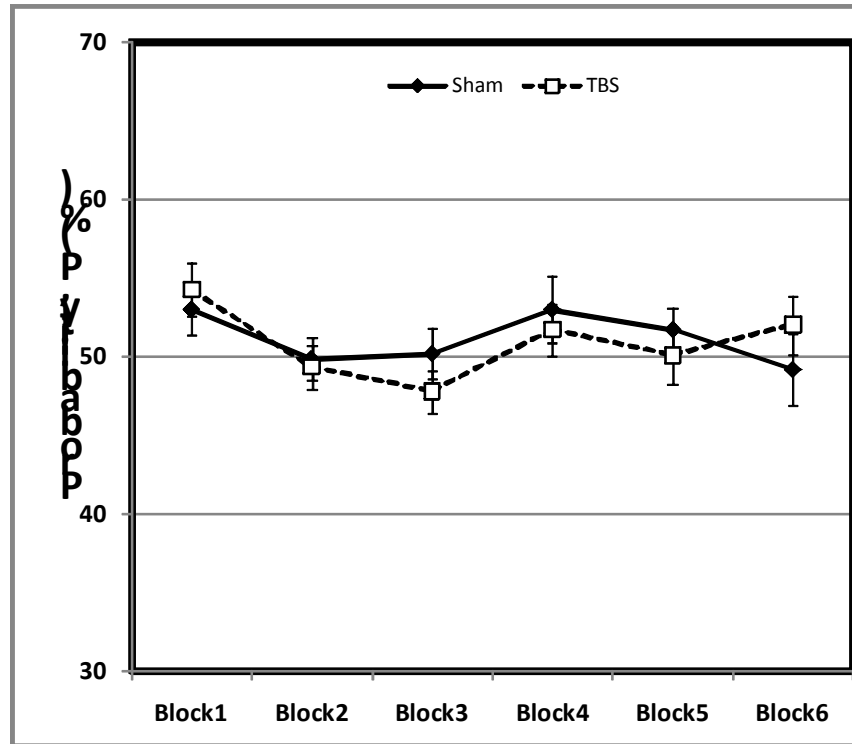


Fig 5.2 Probability of an individual trial being better than previous

trial: There was no change in probability of next movement being better than previous movement across blocks for both TBS (dashed line, unfilled squares) and sham (solid line, filled diamonds) sessions.

Fig 5.2 demonstrates the analysis showing that no underlying learning rule or strategy was being acquired during the task performance as there was no change in variability or probability of improvement. A two-way repeated-measures ANOVA for factors “TBS” and “BLOCK” was performed. There was no effect of the factor “TBS” ($F = 0.049$, $p = 0.830$) and no effect of the factor “BLOCK” ($F = 1.673$, $p = 0.161$). There was also no interaction of “TBS” x “BLOCK” ($F = 0.903$, $p = 0.488$).

This analysis demonstrates that any performance improvement during the ballistic motor learning task was not associated with a progressive increase in probability of performing a better movement suggesting that no underlying learning rule or motor memory was being acquired during this task.

The question then arises as to what mechanism is responsible for enhancing learning after iTBS. Working from first principles, if no learning rule or motor memory was being acquired, the only mechanism driving performance change (whether improvement or deterioration) is intrinsic performance variability. We sought to test the viability for performance variability as a driver of performance change in this task by constructing a simple theoretical model of the ballistic motor learning task.

5.5 Modelling the ballistic motor learning task

A simple theoretical model was designed to show how a performance improvement may be achieved with minimal assumptions about how learning would occur. The aim of creating such a model was to allow us to investigate the effects of altering performance variability on the outcome, and thereby to determine whether it is feasible that such variability may have a positive effect on learning. This model was based on 2 assumptions:

- 1) that there is a maximum physically achievable peak acceleration; and
- 2) that the motor cortex has a fixed repertoire of possible outputs, each coding for different muscle groups, which can be discharged in parallel.

Maximising the motor output would therefore involve determining the optimum weighting in which these motor outputs are to be discharged, presumably favouring task agonists over task antagonists. Thus the system must gradually solve a multi-dimensional problem using feedback given in one dimension, in the form of visual feedback from the previous trial. For the sake of our model we reduced the motor output repertoire to 2 dimensions, represented by 2 orthogonal axes x and y . The contribution of each axis to the observed motor output was defined by the same

exponential function, such that for a given combination (x,y) the observed output is given by:

$$e^{-\left(\frac{x-75}{25}\right)^2} + e^{-\left(\frac{y-75}{25}\right)^2}$$

This function was chosen for the simple reason that it generates a motor output function with a single peak value, achieved at optimum values of x and y (see Figure 5.3a). In our model the following strategy was applied in an iterative algorithm:

- 1) Test the motor output at a random test point centred around the current search-centre coordinates (x,y). The distance of this test point from the current search-centre coordinates obeys a normal probability density function, with a variance that remains fixed (the Output variance, OutputVar).
- 2) If the resulting output is an improvement on the best output so far, then the search-centre coordinates are updated – see step 3. Otherwise these coordinates remain unchanged and the model returns to step 1 (next iteration).
- 3) The search-centre coordinates are moved by a fixed proportional distance along a line joining the current search-centre with the system's perception of the most recent test coordinates. This perception of the test coordinates is not identical to the actual coordinates just used, in order to reflect a degree of error in both recalling the motor output just generated and in interpreting the afferent feedback from the resulting movement. The distance of the perceived test coordinates from the real test coordinates also obeys a normal probability density function with a fixed variance (the Perception variance, PerceptVar). With the new performance coordinates, the model returns to step 1 (next iteration).

We term the extent by which the search-centre coordinates are adjusted (initially 50%) the Learning gain (LearnG), with a higher value denoting a greater degree of motor output change in response to given performance feedback. The Learning gain

thus reflects the capacity for plastic change in this model. It may be noted that the model is simply an iterative implementation of a fixed set of rules – it does not include the capacity to discover underlying rules that find a solution more efficiently.

This model is represented in flow diagram form in Figure 5.3b. The initial test coordinates were always (50, 50), and 100 iterations were performed in the course of each run. The effects of varying either the OutputVar or the PerceptVar were examined by running the model 20 times at each set of values across a range and recording the resulting outputs. For each run of the model, the final output achieved was recorded as the mean of the last 10 trials (out of 100).

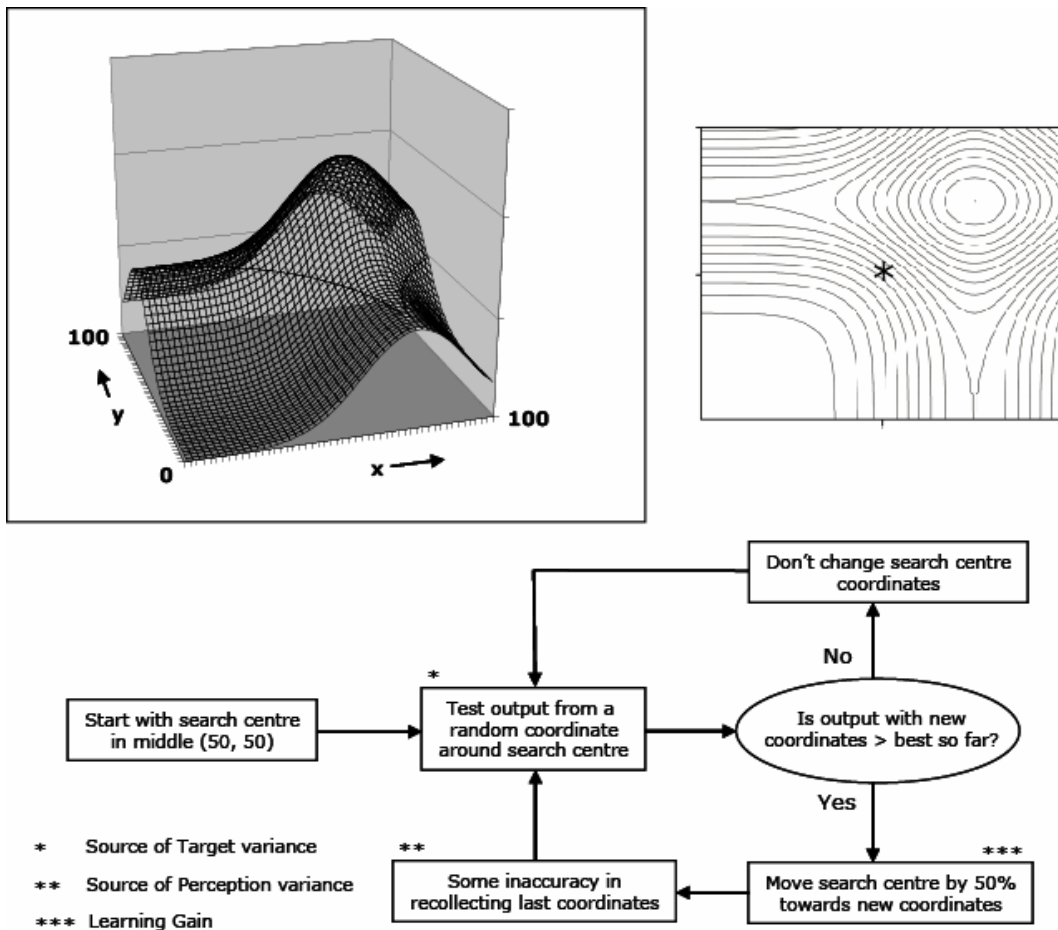


Figure 5.3: Model of a possible strategy for the ballistic learning task

We designed a simple iterative model by which an improvement in performance may be achieved in this task. The aim is to identify the optimum weighting of 2 simultaneously-discharged motor outputs (x and y), using the available performance feedback in the form of observed motor output.

(a) The motor output function employed in the model. The observed motor output (vertical axis) was defined as the sum of the contributions of the 2 individual motor outputs (x and y), each of which obeyed an inverse exponential function such that there is a single peak which represents the maximum possible peak acceleration. In the contour view (right), the asterisk represents the starting position.

(b) The structure of the model is given in flow diagram form. Random combinations of x and y are tried: if the resulting output of a given trial represents an improvement on prior performance then the search centre is moved in the perceived direction of the new coordinate. There are 2 distinct sources of variance, which remain fixed within a given run of the model: Output variance reflects the distribution of trials around the search centre, while Perception variance reflects error in the correct recollection of the previous trial coordinates.

The effects of changing the OutputVar (with PerceptVar set at 1 and LearnG set at 0.2), the PerceptVar (with OutputVar set at 7 and LearnG set at 0.2) and the effects of changing the LearnG (with OutputVar set at 7 and PerceptVar set at 1) are shown in

Figure 5.4. At each setting the final performance depended crucially on the variable tested.

For OutputVar (Figure 5.4a), low values resulted in poor final performance, with little improvement across the 100 trials. Increasing OutputVar was initially beneficial, but beyond an optimal value further increases resulted in impaired performance. For LearnG (Figure 5.4c), increases resulted in a similar inverse-U-shaped curve with an optimum value beyond which further increases were detrimental to performance. For PerceptVar (Figure 5.4b), by contrast, there was no optimal value – increasing this form of variability resulted in a steady decline in performance. Detailed analysis of the interaction between these variables demonstrates that this principle holds true for all values of these variables (Figure 5.4). Thus in this simple model of a learning strategy we observed a complex interaction between variability and learning, with divergent effects of the 2 forms of variability tested on final performance. For OutputVar and LearnG, there is an optimum range where maximal performance gain occurs while any increase in PerceptVar is detrimental.

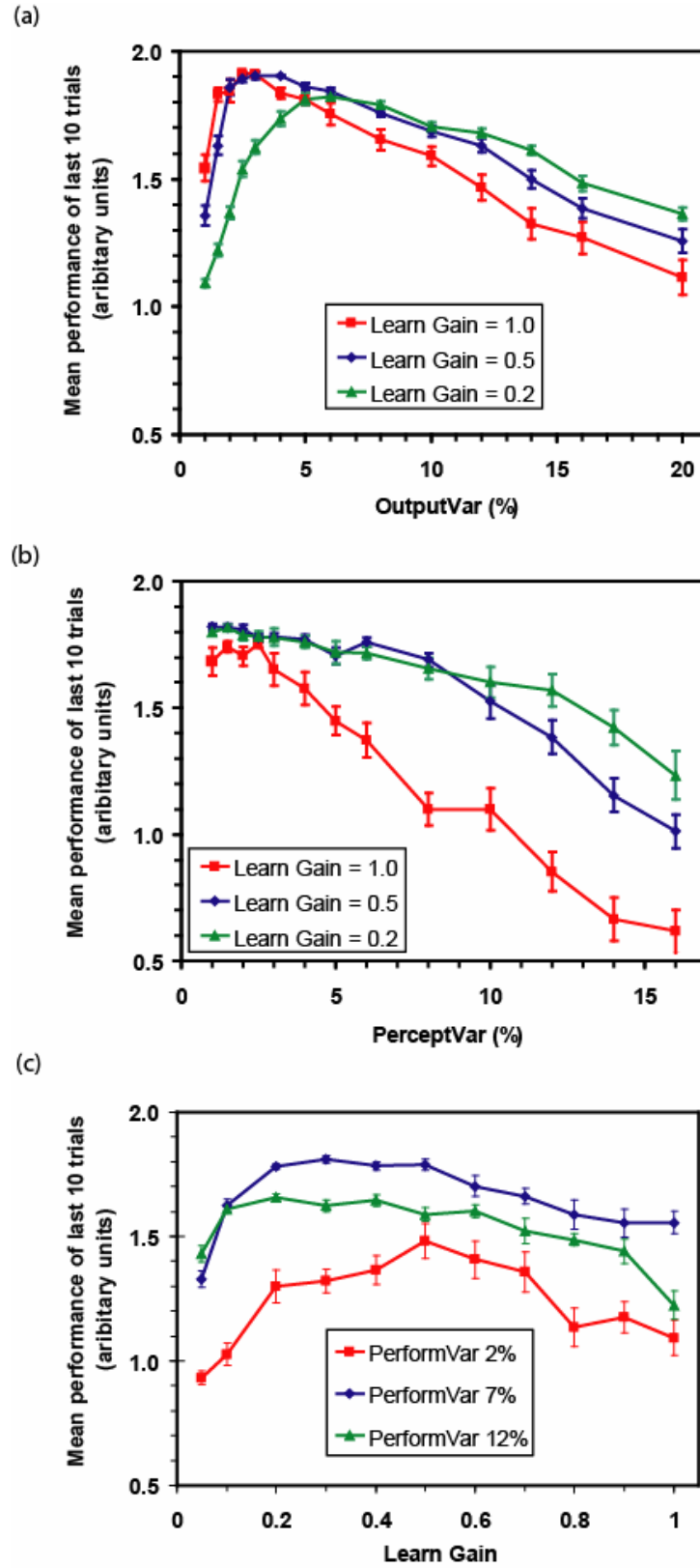


Fig 5.4: The interaction of performance variability, perception variability and learning gain in the ballistic learning model

The model was run 20 times with each setting of learning gain (LearnG), and the final performance recorded as the mean of the last 10 trials (out of 100).

(a) For output variability (OutputVar) the U-shaped curve persists across the 3 values of LearnG tested: with increasing OutputVar learning initially increases and then drops off, such that there is an optimum value of OutputVar for each setting of LearnG. Interestingly, the curves for the low and high values of LearnG intersect.

(b) For perception variability (PerceptVar) the curve is similar across the 3 values of LearnG tested: increasing PerceptVar is consistently detrimental to learning outcome.

(c) For learning gain (Learn Gain) the curve demonstrates a U-shaped tendency which holds true whatever value of OutputVar.

The interaction between LearnG and OutputVar suggests that in this model there is an interactive relationship between plasticity (LearnG) and output variability: when the capacity for plasticity is low then effective learning is favoured by greater output variability, while in the context of greater plasticity less variability is favourable.

5.5.1 Implications of the model

The idea that increasing performance variability may improve learning may initially seem counter-intuitive. However, there are situations in which variability can drive performance change. In our simple model of the task, we assume that the subject is

unable to formulate a set of rules to aid improvement from the information available about the current trial. This contrasts, for example, with sequence learning in which knowledge of the sequence in itself predicts the optimal output on each trial and is validated by the fact that there was no improving probability of learning between blocks within a session.

In the model, the drivers to performance change were random variation in motor output (OutputVar) and in the memory of the previous trial's output (PerceptVar). The effects of altering the two forms of variability were fundamentally different. While variability in accurate recollection of the previous trial (PerceptVar) was entirely detrimental effect to final performance, the same was not true for variability in search-centre coordinates (OutputVar) where an inverted U-shaped curve was observed.

Increasing OutputVar allows the model to try a wider range of combinations, allowing the system to 'escape' a performance plateau and continue improving. This is akin to a selection process where a degree of diversity allows a gradual evolutionary process to occur. On the other hand, excessive OutputVar adversely affects the reproducibility of good movements so that learning suffers. The LearnG determines the extent of output adjustment made in response to an improvement and so reflects the degree of plasticity available. A relatively small amount is required for optimal learning, beyond which improvement declines. Impaired performance at LearnG higher values were explained here by greater system variability, suggesting a complex interaction between plasticity and variability. Thus, a highly variable system would benefit from less plasticity (due to the risk of learning an error) while a less variable system would benefit from greater plasticity.

These results are obtained from a simple model that shows how the motor system might operate in the absence of rule-based optimisations. Although an extreme

example, it does serve to illustrate that a beneficial role for output variability in learning is at least feasible. It is interesting that even in such a simple system there is a clear interaction between variability and plasticity with respect to net performance gain. We sought now to test whether performance variability in our experimental data from experiment 1 is associated with improved performance.

5.6 Analysing variability of performance during learning

To analyse performance variability, a trial-by-trial analysis was performed of each session in order to produce a measure of performance variability for that session. Such a measure needs to reflect variability of performance in relation to an implicit ‘target’ that increases with learning across the 6 blocks. This was calculated iteratively across the approximately 180 trials (30 trials per block, minus trials where subjects missed movements) by measuring the difference between actual performance and a changing ‘target’ defined based on retrospective performance. From trial 2 onwards, each trial performance was tested for whether the performance exceeded the previous ‘target’: if so, then this target was increased by 50% of the difference between the new best outcome and the old target. This reflects the subjective experience of the subject where the subject aims to maximise their performance compared to the memory of what they have achieved and does not impose any preconceived learning rule on the analyses (which any simple curve-fit would do). This value of 50% was chosen in order that the changing target would not be excessively affected by isolated outliers. For each trial, the difference between the performance and the current target was calculated, and the mean and standard deviation of this difference from ‘target’ was determined for the session, and the coefficient of variability was calculated as (standard deviation / mean). This value thus reflects variability of the difference of the

performance from the changing target value, crucially, is unaffected by the magnitude of baseline performance or overall change within a session. An example of a trial-by-trial measure of peak acceleration is provided in Fig 5.5.

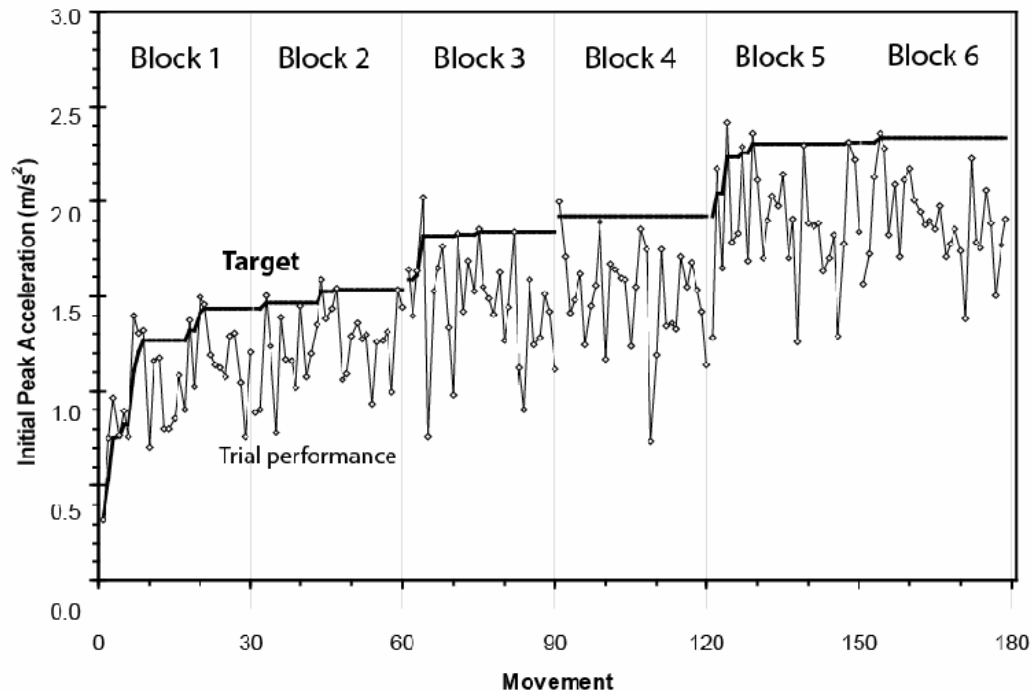


Fig 5.5: Example of a trial-by-trial measure of peak acceleration (diamonds, plain line) with a derived ‘moving target’ (bold line) which increases every time it is exceeded by a new trial. Coefficient of variability is thus derived from the variability of the difference between the trial acceleration and the ‘target’.

Given with placebo, iTBS enhanced both motor learning and corticospinal excitability. The addition of nicotine, however, had differing effects on these 2 parameters, enhancing the MEP increase but blocking improved learning: this divergence implies that the increase in corticospinal excitability is of itself insufficient to explain the positive effect of iTBS on motor learning. We therefore examined the behavioural data from Experiment 1 to determine whether iTBS had any effect on the

variability with which the motor task was performed. For each session, a measure of trial-by-trial performance variability was calculated using a method that is independent of baseline performance or overall improvement. Performance variability was calculated as variability of the difference from a continuously updated target derived from previous good performance. Fig 5.5 shows the target calculated for a single session. This iterative approach made no assumptions about the mechanism a subject learns, and the coefficient of variability (standard deviation / mean) ensured that the resulting measure was independent of performance magnitude.

Figure 5.6a demonstrates that iTBS alone enhances the coefficient of variation in performance which is confirmed on a 2-way ANOVA testing the effects of the factors ‘Stimulation’ and ‘Preparation’, and revealing a significant interaction ($F_{1,9}=7.637$, $P=0.022$), with a significant main effect of Stimulation ($F_{1,9}=9.265$, $P=0.014$) but not of Preparation ($F_{1,9}=0.150$, $P=0.707$). This interaction was explained by significantly greater performance variability after TBS than after sham stimulation in the placebo sessions ($P<0.001$) but not in the nicotine sessions ($P=0.965$). A comparison of the TBS sessions (nicotine vs placebo) revealed that performance variability was significantly reduced in the presence of nicotine ($P=0.031$). The results of this analysis suggest that iTBS had the effect of increasing performance variability when the subject had taken placebo, but this increase in performance variability did not occur if they had taken nicotine: this was the same pattern observed in the effect of TBS on learning.

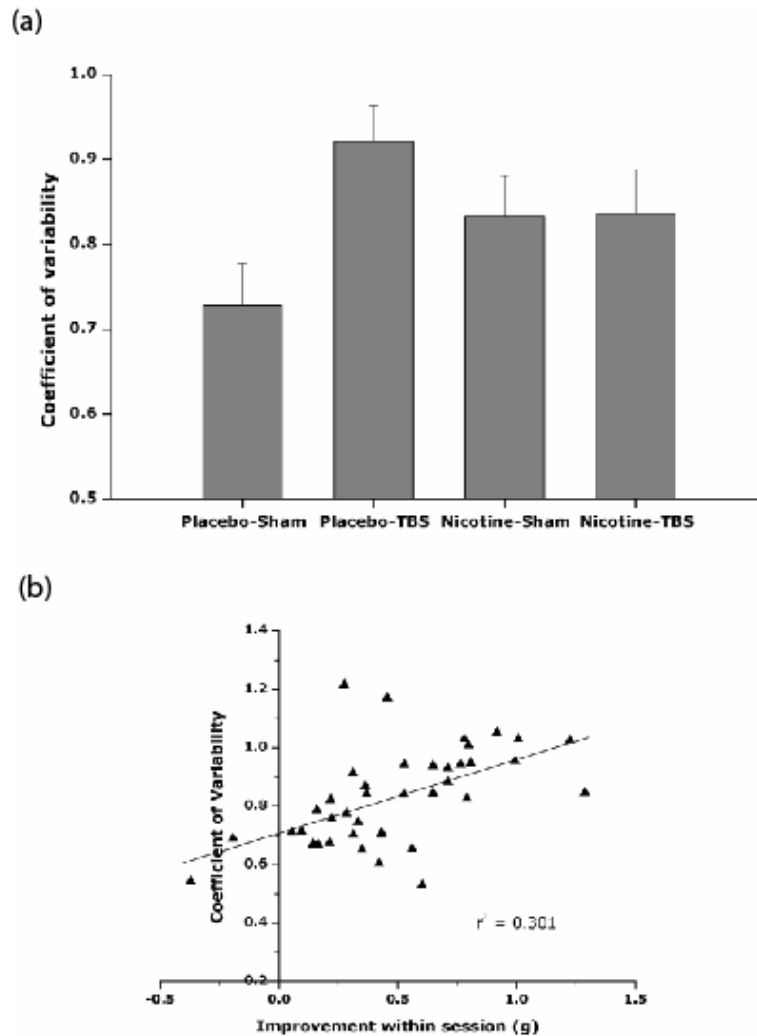


Figure 5.6 Performance variability during the learning task (group means of each session)

(a) The effect of TBS (vs sham stimulation) on variability was different in the nicotine and placebo sessions, as revealed by a significant interaction between the factors Stimulation and Preparation (see text for ANOVA details). In the placebo sessions, performance variability was significantly greater after TBS than after sham stimulation (** $p < 0.001$), whereas TBS did not affect variability in the presence of nicotine ($p = 0.965$). Moreover, in the sessions with TBS

performance variability was significantly reduced in the presence of nicotine ($p=0.031$).

(b) The learning sessions were combined (regardless of session type) in order to examine the relationship between the coefficient of variability and the extent of the total improvement achieved across the 6 training blocks. There was a strong correlation between these variables, such that greater performance variability was associated with greater learning ($r^2 = 0.301$, $p<0.001$).

We further tested whether performance variability and learning were related in these experiments. When these variables were plotted for all 40 sessions regardless of session type (Figure 5.6b) there was indeed a strong positive correlation between performance variability and total learning, defined as (Block 6 mean performance – Block 1 mean performance) ($r^2=0.301$, $P<0.001$). This provides some support for a modulation of this form of variability as a candidate mechanism for the beneficial effects of iTBS on learning observed here.

5.7 Control experiment: TBS on variability of TMS-evoked movements

In order to assess the effect of iTBS on motor output variability in a manner independent of task performance, we measured vectors of thumb movements evoked by a single TMS pulse (Experiment 2). This paradigm is similar to that employed by Classen et al 1998. A triaxial accelerometer (Entran Sensors & Electronics, Les Clayes-sous-bois, France) was placed on the right thumb proximal phalanx, allowing the derivation of a vector for each evoked thumb movement.

We used a stereotactic neuro-navigation system (Brainsight, Rogue Software, Montreal Quebec, Canada) to identify a location in the hand area of the primary motor

cortex at which stimulation produces a TMS-evoked movement of a stable vector, defined by at least 8 out of 10 vectors lying within the same quadrant. After an initial baseline block (20 TMS-evoked movements), iTBS was then delivered to the same location of the motor cortex and a post-intervention block was recorded.

For experiment 3, the concentration parameter (κ) was derived from the TMS-evoked movement vectors using the circular statistics software Oriana (Oriana for Windows, Kovach Computing Services, Anglesey, Wales). κ is a measure of the directionality of the distribution (Fisher, 1993) for which a value of 0 would represent no vector directionality (a distribution resembling a perfect circle), and thus maximal motor output variability.

We first performed the Rayleigh test on the movement vectors in order to verify that they were not circularly uniform. This confirmed that the κ is a valid measure of non-uniformity for this data set. We derived this measure at baseline, after iTBS and after no stimulation. κ is a non-linear parameter and was thus transformed with \log_{10} and a mean calculated for graphical representation. The non-parametric Wilcoxon paired signed ranks test was used to test for significant differences.

Here we tested the effect of iTBS on the directional variability of a TMS-evoked thumb movement, an outcome measure independent of movement magnitude. Figure 5.7a shows data from two representative subjects, in which the direction of movement was considerably dispersed following iTBS but remained stable after no intervention. In Figure 5.7b the change in statistical concentration (κ) of TMS-evoked movement vectors following either iTBS or no intervention is shown for each subject. A lower value for κ denotes a greater degree of variability, so that a negative change in this parameter indicates an increase in movement dispersion. The baseline value for κ did not differ between the 2 session types (Wilcoxon paired signed rank test $P=0.249$). In

the sessions without stimulation κ was not significantly changed at the post-intervention time point (Pre 0.677 ± 0.159 (mean \pm SE); Post 0.849 ± 0.219 ; $P=0.249$). Following iTBS, by contrast, there was a significant reduction in κ (Pre 0.924 ± 0.183 ; Post 0.355 ± 0.183 ; $P=0.046$): iTBS was therefore associated here with an increase in directional variability of the TMS-evoked motor output.

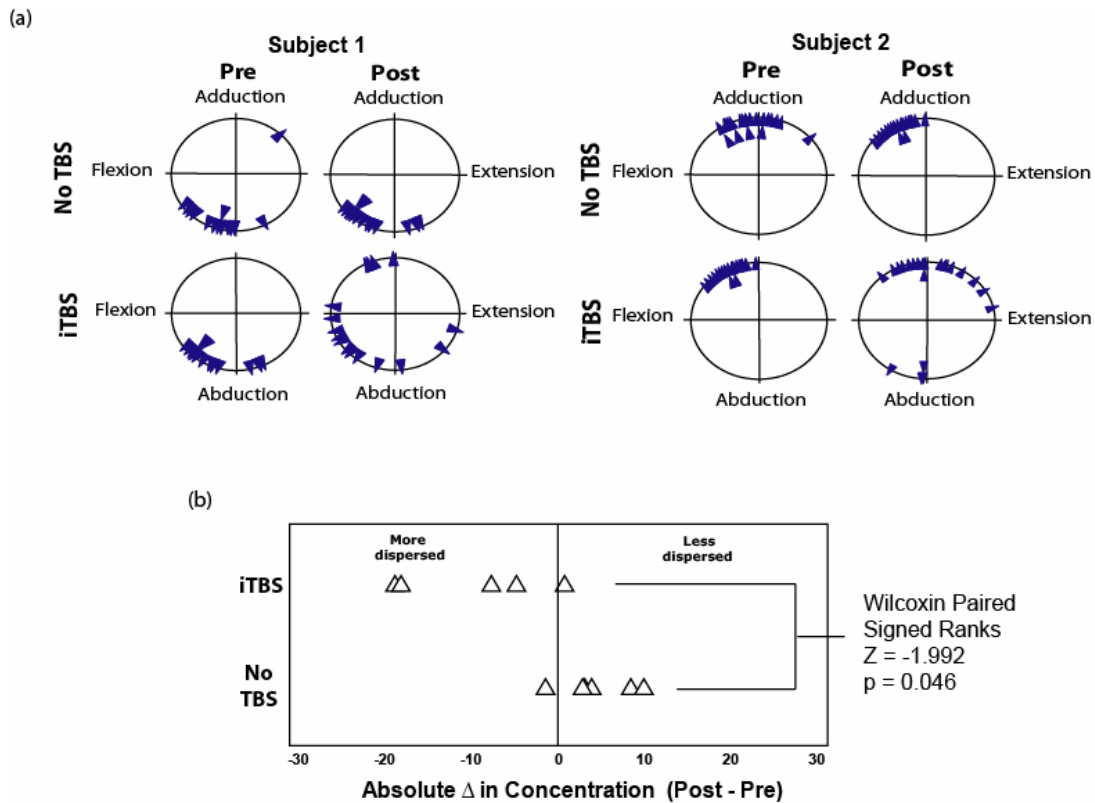


Figure 5.7 The effect of iTBS on the variability of TMS-evoked thumb movements

(a) Directional vectors for 20 consecutive TMS-evoked thumb movements are shown for two representative subjects. At the start of each session, and after no intervention, the direction of thumb movement was stable. Following iTBS the direction of evoked

movements became more variable, such that the concentration parameter (κ) was reduced.

(b) The change in concentration parameter κ following either iTBS or no intervention is shown for each subject. A negative change in κ denotes an increase in the variability of TMS-evoked movement vectors. Baseline values did not differ between the 2 session types (Wilcoxon paired signed rank test $P=0.249$). κ was significantly reduced following iTBS (0.046) but not after no intervention (0.249), indicating that iTBS increased the variability of TMS-evoked thumb movements.

5.8 Conclusion

An analysis of performance variability in Experiment 1 revealed a pattern similar to that observed with learning outcome: iTBS increased variability but this effect was blocked by nicotine. Moreover, performance variability in a given session was correlated with the behavioural gain observed. One source of changes in performance variability may be due to increased variation in the voluntary drive to motor cortex from distant sites. However, the fact that iTBS increased directional variability in Experiment 2, in which a TMS pulse evoked motor cortical output directly, suggests that a major source of change lay within the motor cortex itself. We conclude that iTBS increases motor cortex output variability independent of voluntary drive.

Variability is known to increase with larger outputs (Jones et al 2002, Hamilton et al 2004). This scaling effect is recognised in sensory input from psychophysical literature and follows the Weber–Fechner law:

"In order that the intensity of a sensation may increase in arithmetical progression, the stimulus must increase in geometrical progression."

(translated from Fechner, 1860)

If a Weber-type phenomenon is involved in the motor learning task, it is conceivable that the initial performance (or excitability) may produce a geometric increase in performance or performance variability. Although this is corrected for in the calculation of coefficient of variation by normalisation with baseline, this correction for baseline may not fully account for the increase in performance variability. This could mean that any increased variability may be an epiphenomenon of increased baseline performance (or excitability). It is telling to note that in the TBS-placebo arm of the experiment, the mean performance in Block 1 appears to be larger than the mean performance in other experimental arms, although this is not significant ($p > 0.05$). Thus, small changes in baseline excitability or performance that this study is not powered to detect, may produce large changes in performance variability which is detected in our trial-by-trial analysis. Thus, a causal relationship between motor performance and variability is not established and further experiments will need to be done to clarify the statistical correlation.

Nicotine blocked the iTBS-related increase in performance variability, but did not alter variability on its own. A recent study in humans has suggested that cholinergic stimulation may increase the signal-to-noise ratio in the motor cortex (Kuo et al 2007). Similarly, nicotine increases the gain in thalamic inputs to the visual cortex (Disney et al 2007). The present results may be explained in these terms if nicotine were to reduce variability within the motor cortex, with a consequent negative effect on learning.

In the present study we demonstrate enhanced acquisition of a motor task following iTBS, and blockade of this effect by nicotine. This interaction was not explained by effects on synaptic plasticity, at least as judged by their influence on corticospinal excitability. We believe that iTBS may enhance task acquisition by increasing variability of motor cortical output and thereby driving performance change rather than by increasing synaptic strength although this causal relationship is not proven. We used a simple mathematical model to demonstrate that a beneficial effect of variability on learning is theoretically feasible in a simple task and this model has similarities to differential stochastic motor learning (Frank et al., 2008; Schöllerhorn et al., 2009) and schema motor learning models (Schmidt et al., 1975) in the movement science literature.

The potential to enhance the effects of rTMS pharmacologically has obvious clinical appeal. The positive effect of iTBS alone on subsequent learning may also provide encouragement in this regard, as it raises the possibility of enhancing the response to therapy in patients with motor impairment. However, the observed dissociation between the physiological and behavioural effects of such a combined approach introduces a note of caution. Incorporating concepts with computational or cognitive modelling into the study of motor learning may help to shed light on this relationship.

Chapter 6

Intracortical circuits and practice-dependent plasticity

Work described in this chapter have been published:

Teo JT, Terranova C, Swayne OB, Greenwood R, Rothwell JC. Practice-dependent plasticity is limited by different intracortical circuits. Exp Brain Res. 2009

Mar;193(4):555-63.

6.1 Introduction

Pharmacological interventions coupled with transcranial magnetic stimulation (TMS) methods have made it possible to study a number of inhibitory circuits in the human cerebral cortex and to test how they are involved in particular types of motor behaviour. The present paper focuses on the role of subtypes of the GABAA receptor in synaptic plasticity induced when subjects learn a new motor task.

Butefisch et al. (2000) initially showed that if subjects practise an isolated thumb movement in a particular direction then the amplitude of MEPs evoked in agonist muscles is larger after practice than before. Since this was blocked by NMDA receptor antagonists, it was presumed to involve LTP-like changes in the efficacy of glutamatergic synapses in motor cortex. The authors also found that the effect was blocked by pretreatment with lorazepam, a non-selective GABAA agonist. The latter was compatible with reports in the animal literature that emphasised the role of GABA in regulating motor cortical plasticity (Hess et al. 1996) as well as with other investigations of synaptic plasticity in humans (Ziemann et al. 1998a; Ziemann et al. 1998b; Ziemann et al. 2001; Pleger et al. 2003). Taken together these results suggest that LTP-like plasticity is enhanced when GABA inhibition is reduced. However, there is no information on whether specific subtypes of receptor are preferentially involved in the effect.

The present experiments examined this question by comparing the effects on practice dependent synaptic plasticity of the non-selective GABAA agonist lorazepam with the selective GABAA- $\alpha 1$ receptor agonist, zolpidem. We predicted that as both drugs are GABA agonists then both of them might potentially interfere with plasticity. However, if

the GABAA-alpha1 receptor were not involved then plasticity would be reduced only by lorazepam whereas it would be unaffected by zolpidem.

Simultaneously to this experiment, we also asked which neural circuits might be most involved in controlling levels of synaptic plasticity. A number of inhibitory intracortical circuits have been identified using transcranial magnetic stimulation (TMS): short-interval intracortical inhibition (SICI), short-interval afferent inhibition (SAI) and long-interval intracortical inhibition (LICI) (Kujirai et al. 1993; Wassermann et al. 1996; Tokimura et al. 2000; Sailer et al. 2002). SICI is believed to involve GABAA receptor neurotransmission (Ziemann 2004; Florian et al. 2008) and LICI is believed to involve GABAB receptor neurotransmission (McDonnell et al. 2006; Florian et al. 2008). Studies with the drug zolpidem indicate that the GABAA-alpha1 receptor is associated with the pathway mediating SAI but not with that mediating SICI (Di Lazzaro et al. 2006; Di Lazzaro et al. 2007). We argued that if particular pathways are responsible for controlling plasticity then changes in the amount of plasticity produced by lorazepam or zolpidem would correlate with their effects on SICI, LICI or SAI.

6.2 Study design

The study was structured as a double-blind randomised controlled cross-over trial with two drug arms. 2.5mg lorazepam or 10mg zolpidem was prepared by the pharmacy of the National Hospital for Neurology and Neurosurgery into unmarked containers. A placebo arm would be easily unblinded and would also not control for the effects of sedation so no placebo arm was used. Subjects had TMS measurements at three time points: T1, T2

and T3. The allocated drug was given at the end of T1. TMS measurements at T2 occur 2 hours after drug ingestion coinciding with the peak plasma concentration of lorazepam and zolpidem: lorazepam, 1.5–2.5 hours (Kyriakopoulos et al., 1978); zolpidem, 0.75–2.6 hours (Salva & Costa, 1995). After T2, subjects performed the motor practice task (see below) and after completion of the task, proceeded to have TMS measurements again at T3. The study design is summarised in Fig 6.1a.

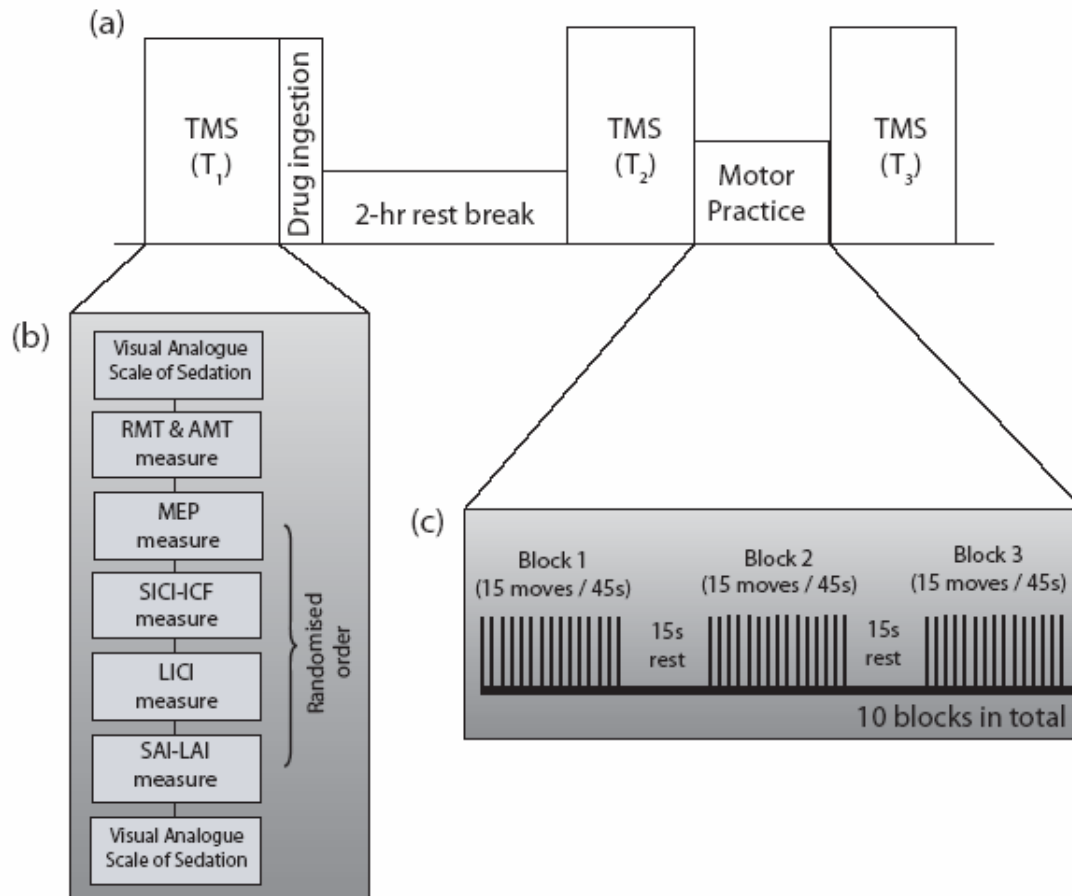


Fig 6.1: Study design of the role of GABA-circuits in practice-

dependent plasticity (a) Timeline of a single experimental session with

three timepoints: T_1 , T_2 and T_3 , corresponding to baseline, during drug peak levels and after task practice; **(b)** neurophysiological measurements were made during each timepoint; and **(c)** task practice consisted of 15 paced thumb abduction movements repeated over 10 blocks with frequent rest breaks.

7 healthy subjects were recruited and informed written consent was obtained. All subjects were right-handed and were not on any medication. For the subjects' second session, they received the other drug. The order was randomised and balanced (4 subjects received lorazepam in the first session). The inter-session interval was 27.7 days (range 10-50 days).

6.3 TMS measurements

Surface EMG was recorded from the left abductor pollicis brevis muscles (APB), the left first dorsal interossei (FDI) and the left flexor carpi radialis (FCR) with Ag/AgCl electrodes using a tendon-belly montage. EMG signals were amplified with Digitimer D360 amplifiers (Digitimer, Welwyn Garden City, UK) with 1000x gain and band-pass filtered (30-1000Hz for MEP) and sampled at 5kHz using a CED1401 laboratory interface and Signal software (Cambridge Electronic Design, Cambridge, UK). Magnetic stimuli were delivered with two Magstim-200 magnetic stimulator (The Magstim Co., Whitland, UK) connected by a Y-cable. A figure-of-8 coil (diameter 80 mm) was adjusted over the optimal scalp position to evoke an MEP in the right APB with the coil handle pointed postero-laterally at a 45° angle to the sagittal plane. The resting motor threshold (RMT) was defined as the lowest intensity capable of inducing at least 5

out of 10 MEPs of $>50\mu\text{V}$ peak-to-peak amplitude. The active motor threshold (AMT) was defined as the lowest intensity capable of inducing at least 5 out of 10 MEPs of $>200\mu\text{V}$ peak-to-peak amplitude during an active tonic contraction of thumb APB.

The settings for the various TMS measures are as follows:

- 1) The corticospinal excitability was measured at rest at 150%RMT over 10 trials.
- 2) Short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) were measured with the test MEP amplitude set at $\sim 1\text{-mV}$ and the conditioning stimulus set at 80%AMT or 100%AMT. The interstimulus interval was 3ms (for SICI), 8ms and 15ms (for ICF). 10 trials were recorded for each condition and the test condition.
- 3) Long-interval intracortical inhibition (LICI) was measured with the test MEP amplitude set at $\sim 1\text{-mV}$ and the conditioning stimulus set at 110%RMT or 120%RMT. The interstimulus interval was 100ms (for LICI). 10 trials were recorded for each condition and the test condition.
- 4) Short-latency afferent inhibition (SAI) and long-latency afferent inhibition (LAI) was measured with the test MEP amplitude set at $\sim 1\text{-mV}$. Electrical stimulation (200 μs pulse width) was delivered to the median nerve using a Digitimer DS7A Constant Current Stimulator (Digitimer, Welwyn Garden City, Herts, UK) at twice or thrice sensory threshold. The interstimulus interval was 22ms (for SAI) and 100ms (for LAI). 10 trials were recorded for each condition and the test condition.

The order of the various TMS measurements were randomised (Fig 6.1b). In addition to the TMS measurements, all subjects filled a visual analogue scale (VAS) of arousal

before and after each time point. The visual analogue scale was a 24cm horizontal line with the left extreme marked 'Wide awake' and the right extreme marked 'Fast asleep'.

6.4 Motor practice

The motor task consists of a paced ballistic thumb abduction task similar to that used in previous studies (Muellbacher et al. 2002). This motor task has been demonstrated to be dependent on the primary motor cortex, is associated with changes in MEP amplitude and movement representation (Classen et al., 1998; Muellbacher et al. 2001) and can be disrupted in the first hour after motor practice (Muellbacher et al. 2002). In this study, the subject's left forearm, hand and fingers were secured in a wooden frame leaving the thumb free to abduct in the horizontal plane. A piezoresistive monoaxial accelerometer (Model SA-105 vibrometer, Fribourg, Switzerland) was attached on the lateral aspect of the left thumb proximal phalanx with the maximal vector being thumb abduction. The accelerometer signal was sampled at 5000Hz and not filtered.

The task consisted of paced ballistic thumb abduction to a loud auditory tone played at 0.333Hz. 15 thumb abductions were performed per block for 10 blocks, with a 15-second rest break between blocks (Fig 6.1c). If the subjects missed any movement, they were required to perform a 'replacement movement'. In total, subjects practiced 150 movements for 10 minutes. Subjects were motivated with verbal encouragement by a blinded investigator. Visual feedback of the acceleration from the previous trial was provided on a computer screen to the subject and the subject was told to attend to the feedback on the computer screen not to their hand as subjective judgement of thumb acceleration is often inaccurate.

6.5 Results

Subjects correctly identified the drug taken on 6 out of 14 sessions (42.9%) which is not above chance (two-tailed Fisher's exact test, $p > 0.05$). The RMT, AMT, sensory threshold and conditioning stimulus intensities at baseline, after drug ingestion and after practice are shown in Table 6.1, 6.2 and 6.3 respectively. There were no statistically significant differences in RMT, AMT, sensory threshold or test stimulus amplitude between the drug arms.

Table 6.1		Lorazepam	Zolpidem
RMT		39.9 + 6.5%	40.0 + 5.2%
AMT		31.1 + 4.5%	29.1 + 5.7%
Sensory Threshold		0.21 + 0.05mA	0.19 + 0.06mA
SICI/ ICF	TS Intensity	49.9 + 8.5%	50.1 + 7.3%
	TS Amplitude	0.64 + 0.35mV	0.85 + 0.29mV
LICI	TS Intensity	49.9 + 0.61%	50.1 + 6.5%
	TS Amplitude	0.61 + 0.4mV	0.79 + 0.27mV
SAI	TS Intensity	50.4 + 8.7%	50.1 + 7.0%
	TS Amplitude	0.74 + 0.53mV	0.98 + 0.15mV

Table 6.1: Subject and TMS parameters at T1 (baseline) where all % values represent % maximum stimulator output unless stated otherwise. Ranges represent standard deviation.

Table 6.2		Lorazepam	Zolpidem
RMT		40.6 + 6.3%	39.9 + 5.6%
AMT		30.4 + 4.4%	30.1 + 5.5%
Sensory Threshold		0.20 + 0.03mA	0.17 + 0.04mA
SICI/ ICF	TS Intensity	52.1 + 8.6%	50.5 + 7.9%
	TS Amplitude	0.66 + 0.38mV	0.78 + 0.29mV
LICI	TS Intensity	53.3 + 8.5%	51.0 + 8.4%
	TS Amplitude	0.76 + 0.68mV	0.73 + 0.27mV
SAI	TS Intensity	52.6 + 8.7%	50.6 + 8.3%
	TS Amplitude	0.52 + 0.29mV	0.71 + 0.31mV

Table 6.2: Subject and TMS parameters at T2 (after drug ingestion) where all % values represent % maximum stimulator output unless stated otherwise. Ranges represent standard deviation.

Table 6.3		Lorazepam	Zolpidem
RMT		40.4 + 7.7%	40.6 + 5.0%
AMT		30.6 + 5.3%	30.0 + 5.6%
Sensory Threshold		0.22 + 0.06mA	0.18 + 0.04mA
SICI/ ICF	TS Intensity	50.6 + 8.8%	50.3 + 7.4%
	TS Amplitude	0.85 + 0.71mV	0.78 + 0.36%
LICI	TS Intensity	50.7 + 8.6%	49.4 + 8.4%
	TS Amplitude	0.82 + 0.7mV	0.72 + 0.3mV
SAI	TS Intensity	51.4 + 8.2%	49.9 + 7.9%
	TS Amplitude	0.66 + 0.50mA	0.66 + 0.33mA

Table 6.3: Subject and TMS parameters at T3 (after training) where all % values represent % maximum stimulator output unless stated otherwise. Ranges represent standard deviation.

6.5.1 Drug-induced changes

The effect of the drugs on MEP amplitudes and various intracortical measures are shown in Figure 6.2. As there were no significant effects of INTENSITY in any intracortical

measures, the graphs represent the mean result of high and low intensity conditioning stimuli for clarity.

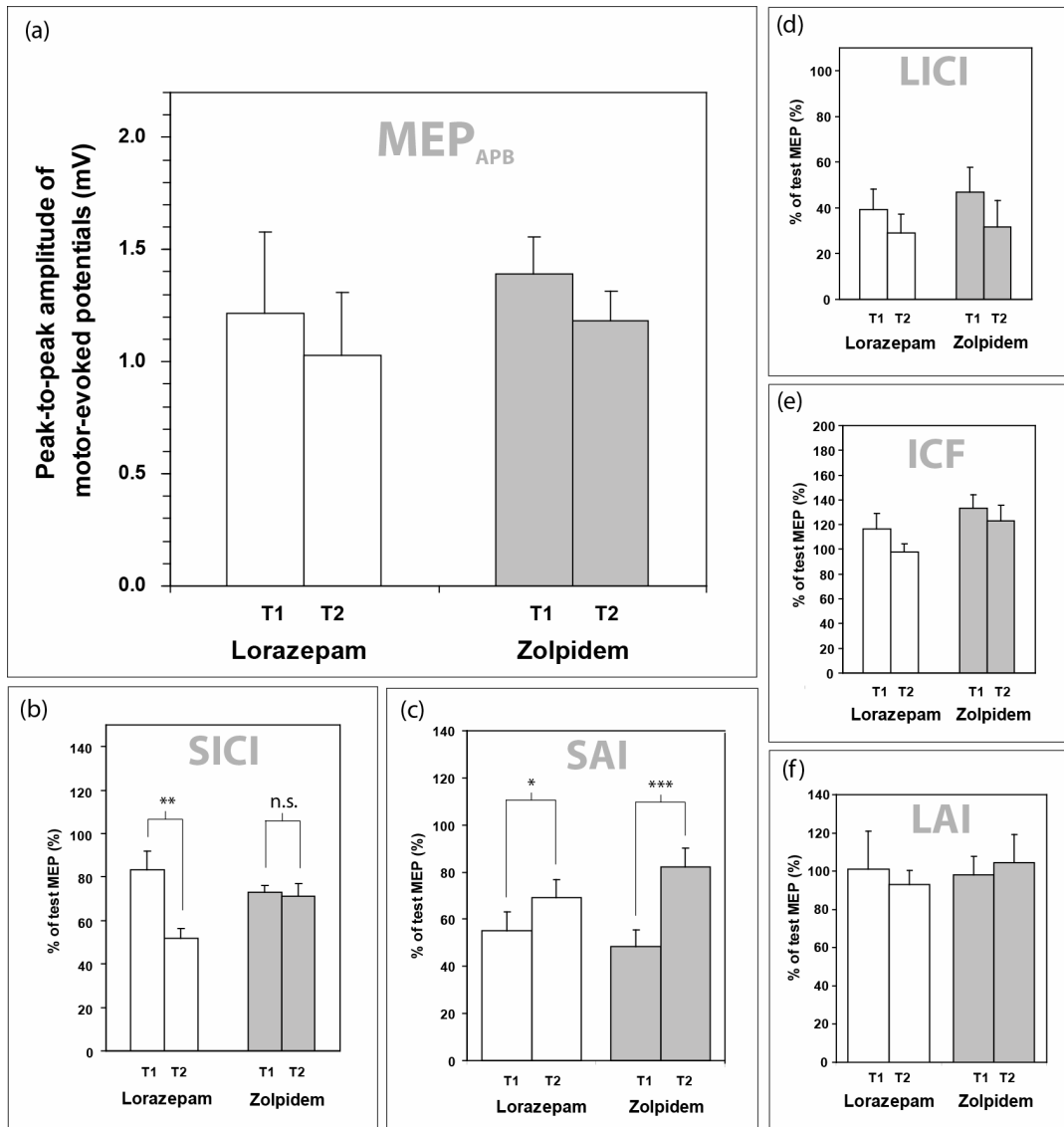


Fig 6.2: The effect of drug on the **(a)** corticospinal excitability in the APB muscle; **(b)** short-interval intracortical inhibition (SICI); **(c)** short-interval afferent inhibition (SAI); **(d)** long-interval intracortical inhibition (LICI); **(e)** intracortical facilitation (ICF); and **(f)** long-interval afferent inhibition (LAI). Unfilled bars represent the lorazepam sessions and filled bars represent the zolpidem sessions. Comparisons were by post-hoc student's

paired t-tests with * represents $p < 0.05$; ** represents $p < 0.01$; *** represents $p < 0.001$ and n.s. represents $p > 0.05$.

There was no significant difference in MEP amplitude after lorazepam and MEP amplitude after zolpidem (Fig 6.2a) with two-factorial ANOVA (within-subject factor TIME and between-subject factor DRUG) showing no effect of TIME ($F_{(1,12)}=0.699$, $p=0.42$), no effect of DRUG ($F_{(1,12)}=0.441$, $p=0.519$) and no DRUG x TIME interaction ($F_{(1,12)}=0.004$, $p=0.950$).

As previously reported (Di Lazzaro et al. 2006), lorazepam increased SICI, while zolpidem did not affect SICI (Fig 6.2b). The difference in effect on SICI of lorazepam and zolpidem was statistically significant on three-factorial ANOVA (within-subject factors INTENSITY, TIME, DRUG) with an interaction of TIME x DRUG ($F_{(1,12)}=7.227$, $p=0.020$). Post-hoc testing with Bonferroni correction showed a significant difference between T1 and T2 for the lorazepam group ($p=0.002$) but not in the zolpidem group ($p=0.802$). Post-hoc testing of the SICI at T2 showed a significant difference between the lorazepam and zolpidem arms (student's paired t-test, $p=0.041$).

For SAI (Fig 6.2c), three-factorial ANOVA (within-subject factor INTENSITY, TIME and DRUG) showed a significant interaction of TIME x DRUG ($F_{(1,12)}=7.23$, $p=0.02$) and no significant interaction of TIME x DRUG x INTENSITY ($F_{(1,12)}=0.206$, $p=0.658$).

Post-hoc testing with Bonferroni correction showed a significant difference between T1 and T2 for the lorazepam group ($p=0.018$) and for the zolpidem group ($p < 0.001$), but no significant differences between the two drugs at T1 ($p=0.539$). Post-hoc testing of the SAI at T2 did not show any significant difference between the lorazepam and zolpidem arms (student's paired t-test, $p=0.118$).

For LICI (Fig 6.2d), three-factorial ANOVA (within-subject factor INTENSITY, TIME and DRUG) showed no significant interaction of TIME x DRUG ($F_{(1,12)}=0.027$, $p=0.871$) and TIME x DRUG x INTENSITY ($F_{(1,12)}=0.041$, $p=0.843$). For ICF at 15ms (Fig 6.2e), three-factorial ANOVA (within-subject factor INTENSITY and TIME; between-subject factor DRUG) showed no significant interaction of TIME x DRUG ($F_{(1,12)}=0.834$, $p=0.379$) or TIME x DRUG x INTENSITY ($F_{(1,12)}=0.031$, $p=0.864$). For LAI (Fig 6.2f), three factorial ANOVA (within-subject factor INTENSITY and TIME; between-subject factor DRUG) showed no significant interaction of TIME x DRUG ($F_{(1,12)}=1.35$, $p=0.268$) or TIME x DRUG x INTENSITY ($F_{(1,12)}=1.07$, $p=0.322$).

In summary after lorazepam, SICI was increased but SAI was decreased while after zolpidem, SICI was unchanged and SAI was decreased.

6.5.2 Practice

All subjects completed 10 blocks of 15 movements training. Peak acceleration during the ten minutes is shown in Fig 6.3. On a two-factorial ANOVA of DRUG x BLOCK, there was an effect of BLOCK ($F_{(1,9)}=2.94$, $p=0.004$) indicating a progressively stronger initial peak acceleration of thumb abduction despite sedation. There appeared to be a fatiguing effect in the zolpidem group after block 7-10 but there were no significant differences between drug sessions ($F_{(1,12)}=0.583$, $p=0.460$) and there was also no significant interaction of DRUG x BLOCK ($F_{(1,9)}=0.868$, $p=0.447$).

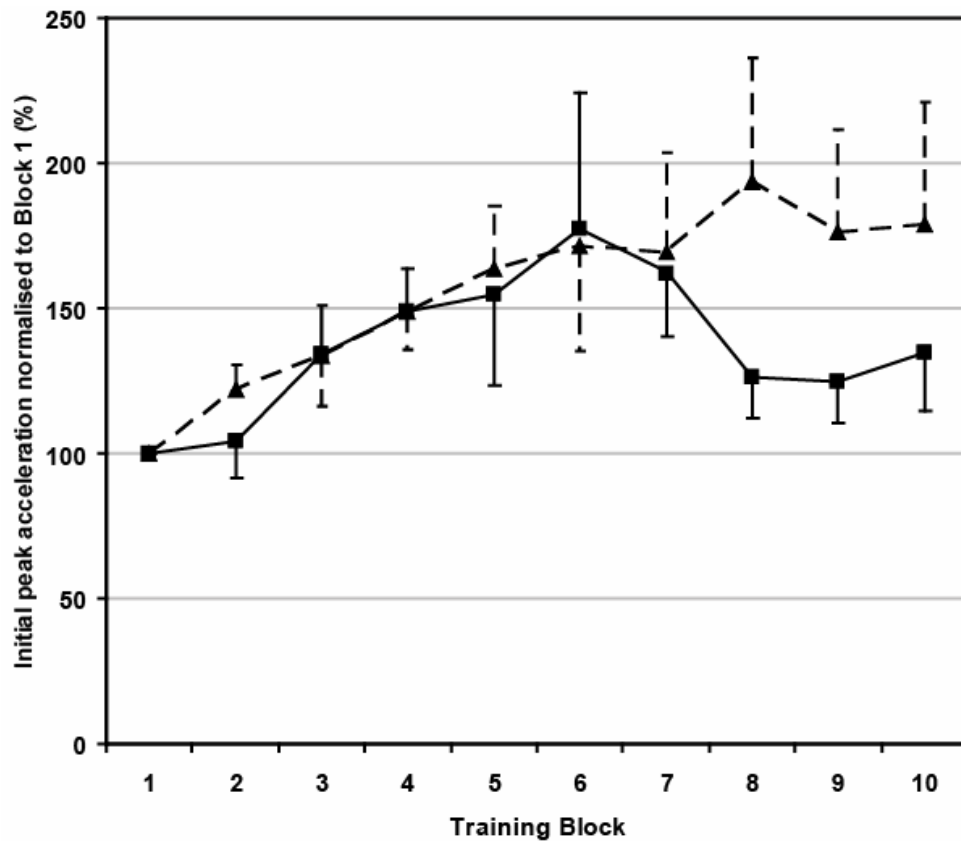


Fig 6.3: Motor performance as represented by initial peak acceleration of thumb abduction over ten 1-minute blocks of practice. In all cases, the solid line and squares represent the zolpidem sessions and the dashed line and triangles represent the lorazepam sessions.

6.5.3 Practice-induced changes

The effect of practice on MEP amplitudes and various intracortical measures are shown in Fig 6.4. As there were no significant effect of INTENSITY in all intracortical measures, the graphs represent the mean result of high and low intensity conditioning stimuli for clarity.

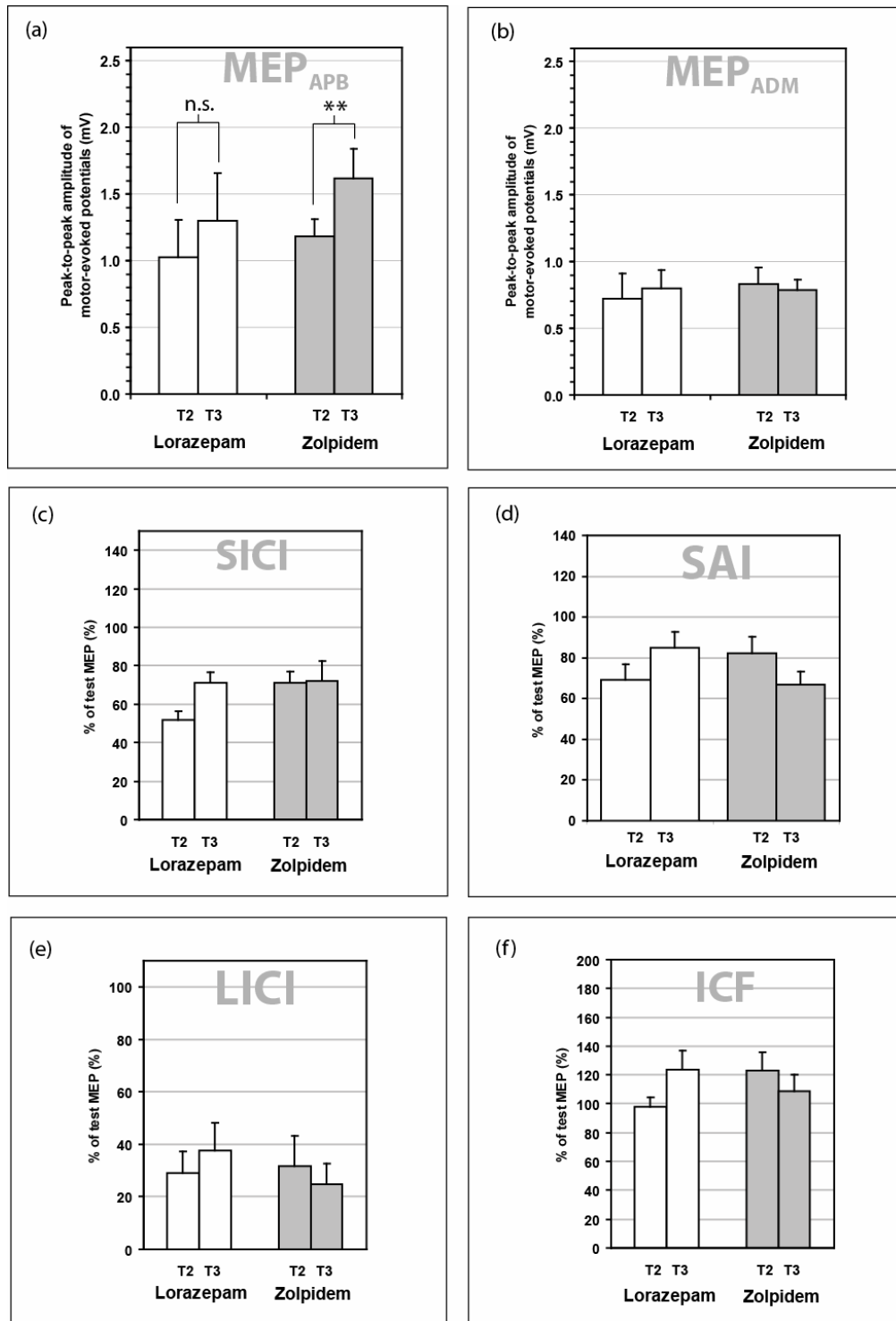


Fig 6.4: The effect of training after drug ingestion on (a) corticospinal excitability in the APB muscle; (b) corticospinal excitability in the untrained muscle, abductor digiti minimi (ADM); (c) short-interval intracortical inhibition (SICI); (d) short-interval afferent inhibition (SAI); (e) long-interval intracortical inhibition (LICI); and (f) intracortical facilitation (ICF). Unfilled bars represent the lorazepam sessions and filled bars represent the zolpidem sessions. Comparisons were by post-hoc paired t-tests with ** represents $p < 0.01$ and n.s. represents $p > 0.05$

The MEP changes after practice are shown in Fig 6.4a and Fig 6.4b and a three-factorial repeated-measures ANOVA was performed with three within-subject factors (TIME, MUSCLE and DRUG). This showed an interaction of TIME x MUSCLE x DRUG ($F_{(1,12)}=5.21$, $p=0.042$). Post-hoc testing with Bonferroni correction showed that in the trained APB muscle, MEP amplitudes enlarged in the zolpidem group ($p=0.005$) but not in the lorazepam group ($p=0.750$). Interestingly, in the untrained ADM muscle of the zolpidem group, there was no significant increase in MEP amplitude ($p=0.634$).

For SICI at 3ms (Fig 6.4c), three-factorial repeated-measures ANOVA (within-subject factor INTENSITY and TIME; between-subject factor DRUG) showed a significant interaction of DRUG x TIME ($F_{(1,12)}=5.40$, $p=0.039$). Post-hoc testing with Bonferroni correction revealed that in the lorazepam arm, there was a significant difference between T2 and T3 timepoints ($p=0.004$) but not in the zolpidem arm ($p=0.841$). However, there were baseline differences at T2 between two drug arms ($p=0.026$) making it likely that this result is a spurious finding resulting from the lorazepam-enhanced SICI at baseline.

For the intracortical measures of LICI, SAI, LAI and ICF at 15ms, repeated-measures ANOVA (within-subject factor INTENSITY and TIME; between-subject factor DRUG in all cases) did not show any significant effect of TIME nor interaction of DRUG x TIME, TIME x INTENSITY or DRUG x TIME x INTENSITY ($p > 0.05$ in all cases).

This is shown in Fig 6.4d-f.

In summary after zolpidem, MEP amplitude in the APB muscle increased after task practice, while after lorazepam, there was no increase after task practice. Other intracortical measures did not show any consistent changes after task practice.

6.5.4 Correlations

The change of SICI from T1 to T2 for the lorazepam sessions (i.e. lorazepam-induced SICI change) was significantly correlated with the change of MEP amplitude from T3 to T2 (Spearman's rank correlation, $\rho = -0.86$, $p = 0.01$) indicating that an increase in SICI was negatively correlated with the amount of plasticity induced. When including data from the zolpidem experimental arm (i.e. where SICI does not change significantly), the correlation is maintained (Spearman's rank correlation, $\rho = -0.574$, $p = 0.032$).

The change of SAI from T2 to T1 (i.e. drug-induced SAI change) was not correlated with the difference of MEP amplitude from T3 to T2 (Spearman's rank correlation, $\rho = -0.04$, $p = 0.94$ for lorazepam sessions and Spearman's rank correlation, $\rho = 0.07$, $p = 0.88$ for zolpidem sessions). This correlation can be seen on Figure 6.5 where positive values of SICI on the right represent stronger inhibition while negative values of SAI on the left represent weaker inhibition.

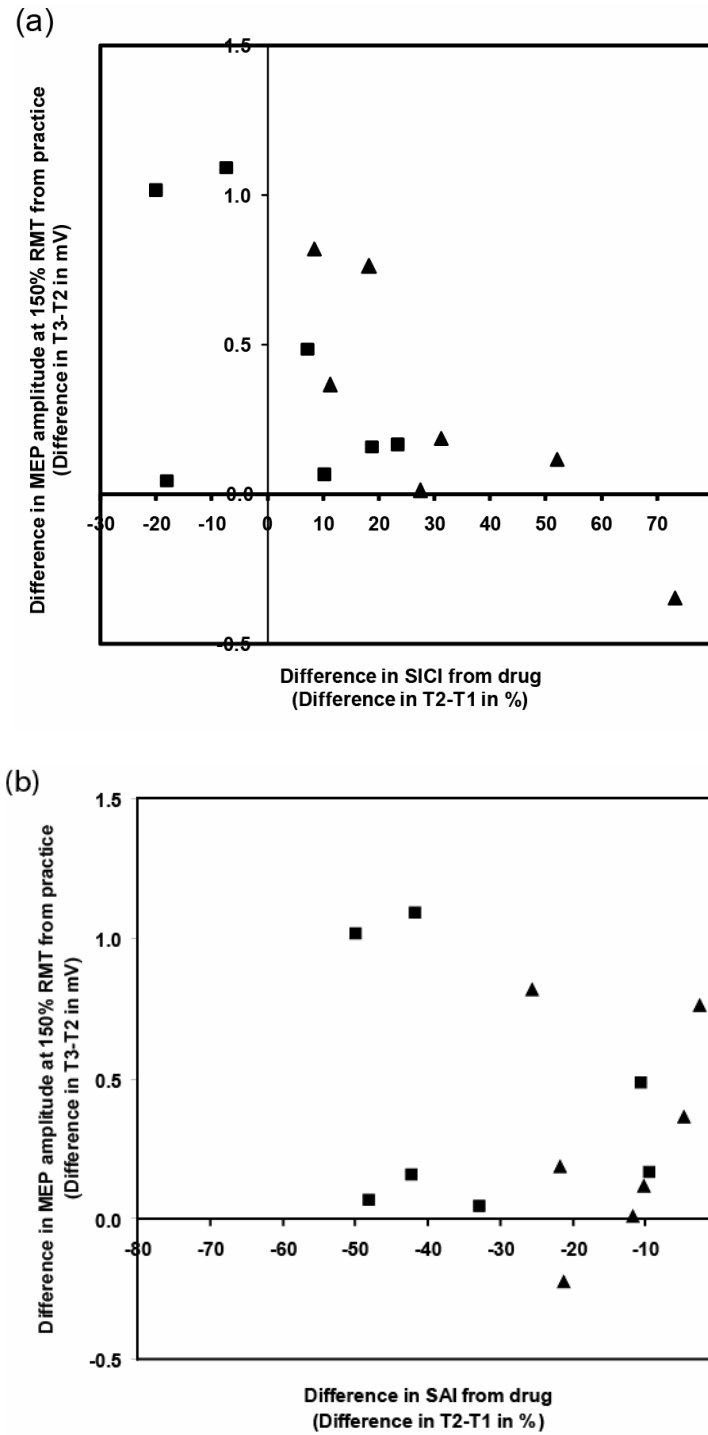


Fig 6.5: (a) Correlation analysis between lorazepam-induced SICI change (triangles) and practice-induced MEP change, zolpidem-induced SICI change (squares) are also shown for comparison; **(b)** correlation analysis

between zolpidem-induced SAI change (squares) and lorazepam-induced SAI change (triangles) with practice-induced MEP change.

Surprisingly, there was also a tendency for drug-induced SAI change to correlate with drug-induced change in the visual analogue scale for the level of sedation (Spearman's rank correlation, $\rho=-0.529$, $p=0.052$). There is no correlation between performance in the task practice and the amount of MEP increase (Spearman's rank correlation, $p=0.34$ for the zolpidem arm of the experiment).

6.6 Discussion

In summary, this study confirms previous reports that non-specific enhancement of GABA_A transmission with lorazepam blocks practice-induced MEP plasticity (Butefisch et al. 2000; Ziemann et al. 2001) and that the selective GABA_A- α_1 receptor agonist, zolpidem reduces SAI but has no effect on SICI (Di Lazzaro et al. 2007). Our novel finding is that zolpidem does not affect practice-induced MEP plasticity. Together the data imply that the SAI circuit is not an important controller of practice-induced MEP plasticity. In contrast, the inhibition of practice-induced MEP plasticity by lorazepam correlates with the increase of SICI induced by lorazepam. This suggests that the circuits involving SICI may be important controllers of practice-induced MEP plasticity.

6.6.1 Drug induced changes in cortical circuits

Our results showing a lack of effect of zolpidem on SICI confirm a previous study (Di Lazzaro et al. 2006). In both studies, the dose of zolpidem was similar, and was calculated to be specific to the GABA_A- α_1 receptor (Mohler et al. 2002; Mohler et al.

2005). The conclusion is that the effect of lorazepam on SICI is not due to activation of GABA_A-alpha₁ receptors. As previously noted (Di Lazzaro et al. 2007), the specificity of the effect on SICI contrasts with that of the SAI circuit which was affected by both lorazepam and zolpidem.

The lack of effect on MEP amplitude by lorazepam in this study compared to previous studies (Boroojerdi et al. 2001; Kimiskidis et al. 2006) can be explained by the low intensity used to assess corticospinal excitability (~60% MSO) compared to previous studies (>65% MSO in Kimiskidis et al., 2006). Additionally, Kimiskidis et al., 2006 also found an effect on cortical silent period (a measure considered analogous to LICI) at higher intensities. Again, higher intensities were not assessed in this current study, so no conclusions can be made about these inhibitory circuits or GABA_B receptors.

6.6.2 Practice-dependent plasticity

This study confirms previous reports that this practice-dependent plasticity is blocked by the GABA agonist lorazepam (Butefisch et al. 2000). Such effects have direct parallels in the animal literature. Direct recordings in the primary motor cortex of rats after motor training suggest that motor training is associated with LTP of the excitatory synapses onto pyramidal neurons in layer II/III of the primary motor cortex (Rioult-Pedotti et al. 1998; Rioult-Pedotti et al. 2000). In addition, induction of LTP in the same synapses by direct electrical stimulation requires GABA activity to be reduced by prior administration of an antagonist, bicuculline (Hess et al. 1996). Thus, it may be that the enhancement of GABAergic activity in humans by lorazepam is the principal cause of the reduction in practice-dependent plasticity (Butefisch et al. 2000).

This conclusion is strengthened by the new data reported here showing that the subjects in whom lorazepam increased SICI were less likely to show practice-dependent plasticity of MEPs than those in whom the drug-induced effects on SICI were weak. The fact that zolpidem had little effect on SICI and little effect on plasticity may even indicate that the inhibitory connections activated during the SICI paradigm are a primary controller of practice-dependent MEP changes. In contrast the inhibitory effects produced by the SAI circuit may be much less relevant, since the changes in SAI were not correlated with changes in plasticity. It would also be consistent with the fact that administration of scopolamine, a muscarinic antagonist that reduces SAI (Di Lazzaro et al. 2000) also reduces (rather than increases) practice-dependent plasticity (Sawaki et al. 2002). Since scopolamine does not affect SICI, its influence on learning is probably via a different mechanism to the GABA-related effects discussed here.

One important drawback of this study is the inability to use a true placebo due to the behavioural effects of these two sedative drugs. Without the placebo arm, it is not possible to quantify precisely how much of the inhibition of practice-dependent plasticity is related to drowsiness and how much is related to GABA-ergic agonism as plasticity is known to be affected by attention (Stefan et al., 2002). Additionally, without a placebo arm, it is not possible to conclusively state that there is no role to play for GABA_A-alpha₁ receptors or SAI in practice-dependent plasticity as there might still be a smaller degree of inhibition of practice-dependent plasticity. Nonetheless, it is possible to conclude that there is a difference in degree of inhibition of practice-dependent plasticity between SICI and SAI.

It is interesting to note that although there was no significant MEP enhancement in the lorazepam arm of the experiment after practice, there was clear improving motor performance during the practice. Thus, additional factors or mechanisms beyond practice-dependent plasticity of MEPs are likely to be playing a role in the performance improvement in the lorazepam arm.

Although this study has shown an association for SICI in practice-dependent plasticity like previous studies (Ziemann et al., 1998; Ziemann et al., 2002), it is also possible that the correlation between the practice-dependent plasticity and the drug-induced effect on SICI may not necessarily be causally linked as lorazepam may be enhancing GABA-ergic signalling in other independent circuits which have yet to be identified and it is these circuits that are predominantly inhibiting practice-dependent plasticity. This remains a possibility although a less parsimonious one.

Finally, why is it that increasing GABAergic signalling in the SICI pathway affects practice-dependent plasticity to a greater degree than SAI? Any inhibition should reduce excitability in the cortex, and make it more difficult to produce LTP (Hess et al. 1996; Glazewski et al. 1998; Steele and Mauk 1999; Casasola et al. 2004). However inhibitory synapses have different effects depending on the spatial localisation of the synapses on the pyramidal cell with inhibitory synapses to the perisomatic region being more potent but less selective than inhibitory synapses to the distal dendrites (Miles et al. 1996, Segev and Burke 1998; Markram et al. 2004). The predominant expression of zolpidem-sensitive GABA receptors to the perisomatic region of pyramidal cells (Klausberger et al. 2002; Xiang et al. 2002) and zolpidem-insensitive GABA receptors to the distal dendrites

(Ali and Thomson 2008) suggest that SICI and SAI may synapse at different locations on the pyramidal cell and thus modulate practice-dependent plasticity to differing degrees. As muscarinic receptor antagonists also affect SAI (Di Lazzaro et al. 2000), SAI inhibitory interneurons may be similar to fast-spiking basket cells described in animal studies which also express GABA_A- α_1 receptor and muscarinic acetylcholine receptors and synapse onto the perisomatic region of the pyramidal cell (Nusser et al. 1996; Kawaguchi and Kubota 1997; Fritschy et al. 1998; Kubota and Kawaguchi 2000; Klausberger et al. 2002; Kawaguchi et al. 2006; Ali and Thomson 2008; Ascoli et al. 2008) and thus the perisomatic inhibition by SAI may be less relevant to practice-dependent plasticity which predominantly occurs in layer II/III synapses in the pyramidal dendrites (Riout-Pedotti et al. 1998; Riout-Pedotti et al. 2000). Thus, the similarities between SAI and inhibition by fast-spiking basket cells are suggestive, but this still remains unproven.

6.6.3 Link between SAI and perception variability

It is tempting to speculate about a link between SAI and PerceptVar described in the model proposed in Chapter 5. PerceptVar represents the accuracy of performance perception so is likely to incorporate sensory elements including somatosensory afferent input (as well as visual input) and spatial cognition (e.g. amplitude comparison) as well. Thus this study's finding that SAI does not appear to have a significant role in practice-dependent plasticity is in line with the model demonstrating that PerceptVar is detrimental to motor learning.

6.6.4 Conclusion

In summary, we demonstrate that practice-dependent plasticity of MEPs is limited to differing degrees by different GABA-ergic intracortical circuits. The use of GABA-subunit selective drugs allows for dissection of the physiological functions of various inhibitory intracortical circuits.

Chapter 7

Intracortical circuits and transcallosal pathways

Work described in this chapter have been previously published:

Avanzino L, Teo JT, Rothwell JC. Intracortical circuits modulate transcallosal inhibition in humans. J Physiol. 2007 Aug 15; 583(Pt 1):99-114.

7.1 Introduction

The role of intracortical circuits in modulating the activity of corticospinal neurons is well-established and can be explored using paired-pulse paradigms as in Chapter 6. The paired-pulse paradigm, short-latency intracortical inhibition (SICI), is believed to represent inhibitory interneuron input onto corticospinal neurons while another paired-pulse paradigm, short-interval intracortical facilitation (SICF), occurring at fixed intervals (Tokimura et al 1996; Ziemann et al 1998), probably represents short-interval summation of excitatory I-wave interneuron input onto pyramidal neurons (Tokimura et al 1996; Di Lazzaro et al 1999; Ziemann et al 1998).

In addition to the projections along the corticospinal tract (CTS), the primary motor cortex also sends projections transcallosally to the contralateral primary motor cortex (Wassermann et al 1991; Ferbert et al 1992; Meyer et al 1995, Gerloff et al 1998). Transcallosal output can be measured either as a period of silence in ongoing EMG activity (ipsilateral silent period; iSP) or as an inhibition of the amplitude of the cMEP evoked by a TMS pulse over the M1 of the contralateral hemisphere (interhemispheric inhibition; IHI). Both these effects have been shown to be absent in some patients with callosal lesions or agenesis of the corpus callosum, as such they are believed to involve transcallosal pathways (Rothwell et al 1991; Meyer et al 1995; Meyer et al 1998; Boroojerdi et al 1996).

Recently Trompetto et al 2004 provided evidence that transcallosal output receives a SICI-like projection suggesting that transcallosal pyramidal neurons are similarly controlled by inhibitory interneurons as corticospinal pyramidal neurons. This chapter tries to characterise further the intracortical circuits synapsing onto transcallosal pyramidal neurons, by looking for a SICF-like effect in transcallosal pathways and determining if these intracortical circuits are also affected by rTMS.

7.2 Intracortical circuits that modulate transcallosal inhibition

7.2.1 Study design

As the relationship between the ipsilateral silent period (iSP) and interhemispheric inhibition (IHI) are not clear, both paradigms were examined in two separate sub-experiments: experiment 1a for iSP and experiment 1b for IHI (Fig 7.1).

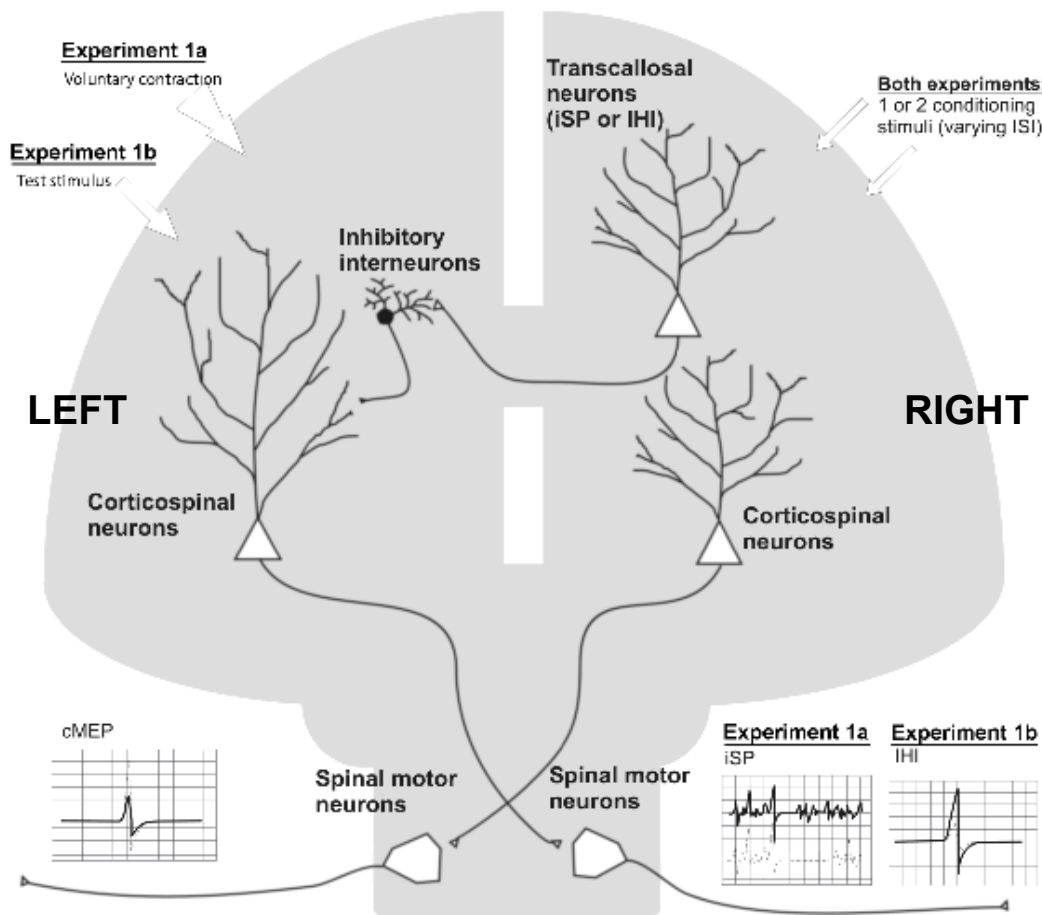


Fig 7.1: Study design of experiment 1a and experiment 1b. A single or pair of equal intensity conditioning pulses were delivered to the right M1 during either a voluntary contraction of the right hand (to measure iSP in experiment 1a) or 40 ms before a test stimulus is delivered to the left M1 (to measure IHI in experiment 1b) and recordings were made from FDI muscles in both hands.

For experiment 1a, we studied 13 right-handed subjects (5 males and 8 females, mean age 29.2 ± 2.9 years, range 25-39 years). During experiment 1a, the subjects maintained a contraction of the left FDI at approximately 50% of maximum voluntary contraction (MVC) while the corresponding right FDI remained at rest. The level of contraction was controlled by visual feedback of ongoing EMG activity of the left FDI muscle. Single or paired TMS pulses delivered using a pair of linked Magstim 200² to the left primary motor cortex were then delivered at varying interstimulus intervals (ISIs) thereby allowing the simultaneous assessment of MEPs in the right FDI (from corticospinal stimulation) and iSPs in the left FDI from transcallosal stimulation.

Paired-pulse stimulation was performed following a paradigm similar to that described by Tokimura et al. (1996) in order to investigate the excitatory interactions that occur at short intervals between two stimuli of identical intensity. To study the iSP, the intensity of the stimuli was set at an intensity that is close to “threshold” for the iSP; for most subjects this was around 150% AMT for both stimuli. Although the intensity used for both stimuli was higher than the one used by Tokimura et al. (1996), a recent study by Ilic et al (2002) showed that this also could produce SICF. Nine interstimulus intervals (ISI) were studied (1.3 ms, 1.5 ms, 2.0 ms, 2.3 ms, 2.5 ms, 3.0 ms, 3.3 ms, 3.5 ms and 4.3 ms). Blocks of 30 trials were performed consisting of three randomly intermixed conditions (10 trials for each condition: CS1 given alone and CS1 preceded by CS2 at two different intervals). The three stimulation conditions (CS1, CS2-CS1 at two different ISI) were tested in a pseudo-randomised order within each block. An example of one subject’s testing is presented as follows:

ISI Test Block	CS1	CS2-CS1 ISI	CS2-CS1 ISI
1	Tested	1.5ms tested	2.0ms tested
2	Tested	1.3ms tested	2.5ms tested
3	Tested	3.0ms tested	4.3ms tested
4	Tested	2.3ms tested	3.3ms tested
5	Tested	1.0ms tested	3.5ms tested

The reason that all ISIs were not tested in one testing block together with TS and CS1-TS trials was because this would result in a very long testing block (10 conditions x 10 trials each) with associated fluctuating levels of relaxation and brain state.

For experiment 1b, we studied 9 right-handed subjects (4 male and 5 female, mean age 32.0 ± 4.9 years, range 26-38 years). A conditioning-test design with a test stimulus preceded 40ms before by either zero (TS), one (CS1-TS) or two (CS2-CS1-TS) conditioning stimuli of identical intensity to the opposite hemisphere with varying interstimulus intervals (ISI) between CS2 and CS1 (Fig 7.1). Interhemispheric inhibition with ISI of 40ms was chosen as this is believed to be more similar to the ipsilateral silent period than interhemispheric inhibition with shorter ISI (Chen et al. 2003). Transcranial magnetic stimulation (TMS) was delivered using a pair of linked Magstim 200² being used for the ‘conditioning hemisphere’ and a single Magstim 200 for the ‘test hemisphere’.

The ISI for CS1-CS2 measured in experiment 1b were 1.3ms, 1.5ms, 2.0ms, 2.5ms, 3.0ms, 3.5ms and 4.3ms similar to that described by Tokimura et al. (1996). For each ISI, the four stimulation conditions (TS, CS1-TS and CS2-CS1-TS at 2 randomly determined ISIs) were tested in a pseudo-randomised order within a testing block. An example of one subject’s testing is presented as follows:

ISI Test Block	TS	CS1-TS	CS2-CS1 ISI and TS	CS2-CS1 ISI and TS
1	Tested	Tested	1.5ms tested	2.0ms tested
2	Tested	Tested	1.3ms tested	2.5ms tested
3	Tested	Tested	3.0ms tested	4.3ms tested
4	Tested	Tested	3.3ms tested	3.5ms tested

The reason that all ISIs were not tested in one testing block together with TS and CS1-TS trials was because this would result in a very long testing block (10 conditions x 10 trials each) with associated fluctuating levels of relaxation and brain state.

Both coils were used at an orientation to produce an antero-medial current over both M1. Intensity of test coil stimulation (TS) was set to produce a cMEP amplitude of ~1.5 mV and the intensity of conditioning coil stimulation (CS1 and CS2) was set to 100% RMT. This level of stimulation was chosen so that neither IHI nor the evoked cMEP were saturated.

7.2.2 Data analysis

7.2.2.1 Data analysis of iSP

The method of analysing iSP has been described in previous work (Trompetto et al 2004). Briefly, the onset and the end of the iSP was assessed in the trace obtained from the average of the ten rectified EMG traces for each condition. The iSP onset was defined as the point after the cortical stimulation at which EMG activity became constantly (minimum duration of 10 ms) under the mean amplitude of EMG activity preceding the cortical stimulus (mean EMG). The iSP end was defined as the first point after iSP onset at which the level of EMG activity regained the mean EMG. The experimenter was not blinded to the condition during analysis and this was not considered necessary as the measurement of iSP used a standardised method which has been previously used (Trompetto et al 2004) as follows:

$$\text{iSP duration} = [\text{time of iSP end}] - [\text{time of iSP onset}]$$

For each condition, the area of the iSP was calculated, using the following formula:

$$\text{iSP area} = ([\text{mean EMG}] \times [\text{iSP duration}]) - [\text{au_iSP}]$$

Then, the iSP area was normalised against the level of contraction using the formula:

$$\begin{array}{l} \text{iSP area} \\ \text{(normalised to contraction)} \end{array} = \frac{[\text{iSP area}]}{[\text{area under mean EMG preceding stimulus}]}$$

Finally, in experiment 1a to compare the conditioned iSP against test iSP, the absolute difference between the normalised iSP area in both responses was used. This simple subtraction was used because it is unknown whether iSP summates linearly or non-linearly.

7.2.2.2 Data analyses of IHI

Peak-to-peak amplitude of MEP contralateral to the test stimulus (i.e. ipsilateral to the conditioning pulses, CS1 and CS2) was measured. Again, it is not known if the IHI would summate linearly or non-linearly. As such, conditioned IHI was analysed as raw peak-to-peak amplitude and absolute difference of peak-to-peak amplitude to unconditioned IHI.

7.2.2.3 Data analyses of cMEP

Peak-to-peak amplitude of cMEP contralateral to the conditioning pulses (CS1 and CS2) was measured. Although it is standard practice to normalise the conditioned cMEP to double the unconditioned cMEP to demonstrate SICF (Ziemann et al 1999; Tokimura et al 1998), it is not known if the facilitation is a linear or non-linear phenomenon particularly at the higher intensities used. As such, conditioned cMEP, like IHI was analysed as raw peak-to-peak amplitude and absolute difference of peak-to-peak amplitude to unconditioned cMEP.

7.2.2.4 Statistical analysis

For experiment 1a and 1b, two-factorial repeated-measures ANOVAs with factors “CS MODE” (CS1 and CS2-CS1) and “ISI BLOCK” (1.3ms, 1.5ms, 2.0ms, 2.3ms 2.5ms, 3.0ms, 3.3ms, 3.5ms and 4.3ms) were performed for iSP, IHI and cMEP on the raw peak-to-peak MEP amplitude or absolute iSP area. One-factorial repeated-measures ANOVA with factor “ISI BLOCK” (1.3ms, 1.5ms, 2.0ms, 2.3ms 2.5ms, 3.0ms, 3.3ms, 3.5ms and 4.3ms) was also performed with all the various methods of correction for cMEP, IHI and iSP (i.e. absolute differences between conditioned and baseline measures or normalised to baseline measure). For post-hoc comparisons, Tukey least significant difference (LSD) test was used for all the various methods of correction for cMEP, IHI and iSP.

Correlations between conditioned IHI with the cMEP were calculated by Pearson linear correlation representing conditioned IHI and conditioned cMEP as absolute difference or normalised.

Any correlations of conditioned cMEP with conditioned IHI or iSP were performed comparing equivalently corrected values (i.e. normalised cMEP with normalised IHI, absolute difference cMEP with absolute difference IHI). Also for correlating the change in iSP area or duration with the change in the cMEP amplitude, only data from subjects in which the test iSP was reliably present from the test stimulus.

7.2.3 Results

None of the participants reported any adverse effect during the course of the study.

7.2.3.1 Demonstration of SICF-like effects on iSP

The intensity of the TMS pulses was adjusted in each subject so that CS2 alone produced a just visible iSP in the contracting FDI muscle. CS1 was applied at the same intensity. The

mean intensity of the stimuli used was $44\% \pm 10\%$ of the maximum stimulator output, or $156\% \pm 11\%$ AMT. We measured the area and duration of the iSP as well as the amplitude of the MEP evoked by CS1 alone in the relaxed contralateral FDI (iSP_{CS1} , $cMEP_{CS1}$) and when conditioned by CS2 ($iSP_{CS2-CS1}$, $cMEP_{CS2-CS1}$).

Fig 7.2 illustrates an example of the interaction between two stimuli of equal intensity delivered at different ISI on the cMEP and the iSP in a representative subject. It is

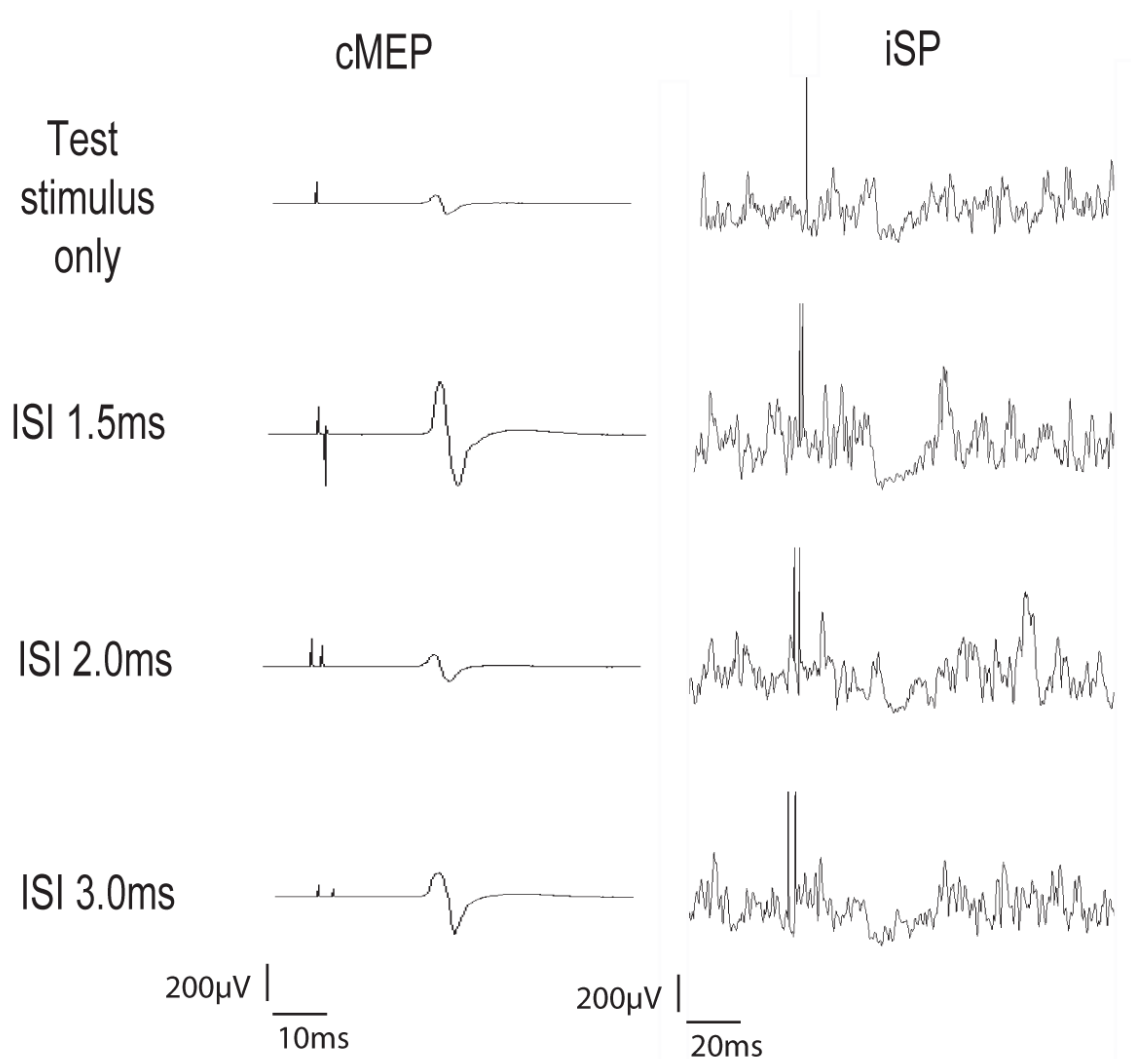


Fig 7.2: Effect of two stimuli delivered at different ISI (1.5, 2 and 3 ms) on the contralateral MEP in the right relaxed FDI muscle (*left panel*) and on the iSP in the left FDI muscle during a contraction equal to almost 50% of the maximal voluntary contraction (*right panel*) in experiment 1a. The first line represents the control response (due to the test stimulus alone) in the contralateral and ipsilateral muscle, while the other three lines represent the responses due to a paired stimulation (test stimulus + conditioning stimulus at 1.5, 2.0 and 3.0ms interstimulus intervals).

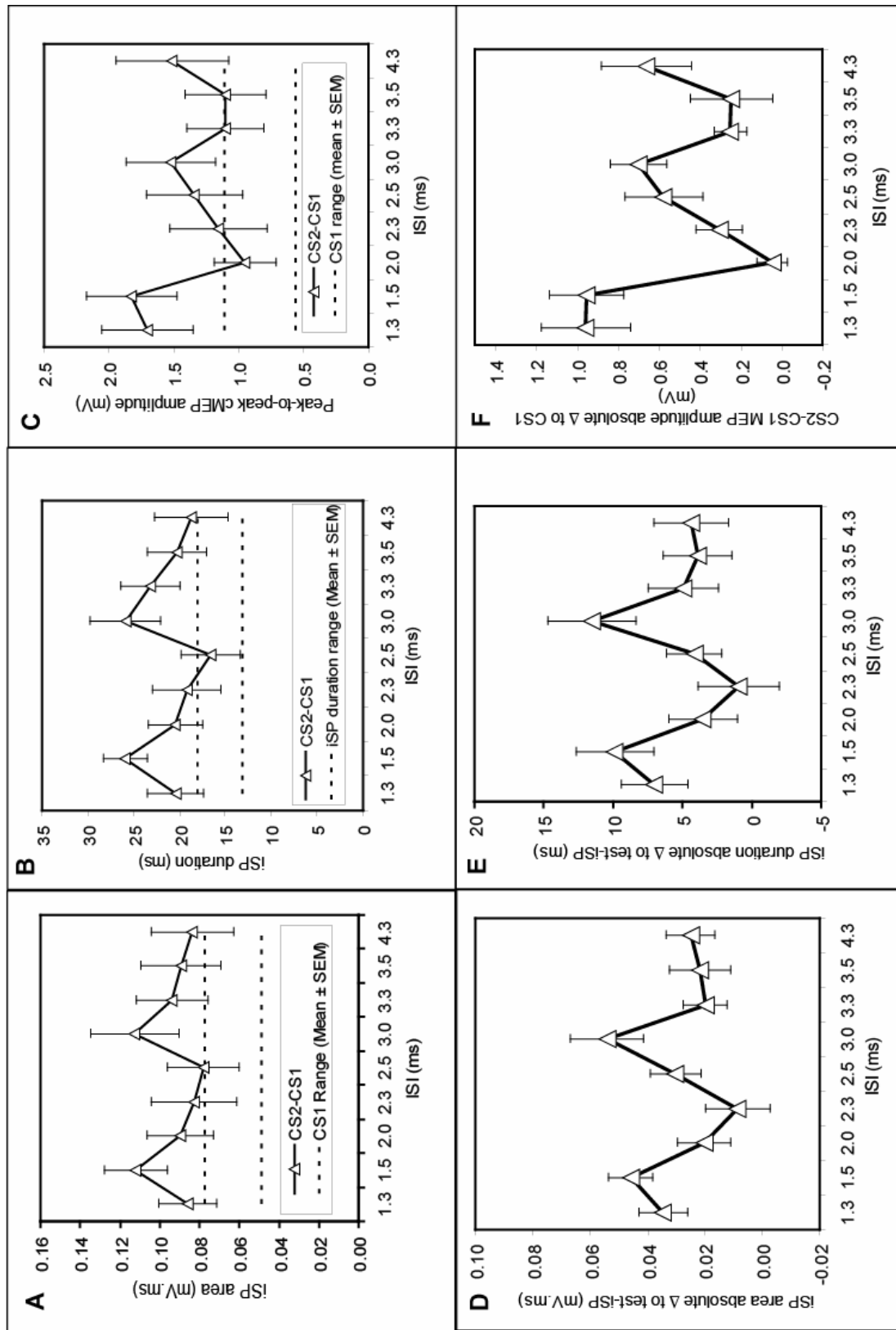
clear that at specific intervals for both the cMEP and the iSP, the response to pairs of stimuli was depended on the interstimulus interval between conditioning stimuli CS2 and CS1.

Due to the difficulty determining the most suitable way to represent conditioned-iSP to the test-iSP, various methods of analysis were used, as follows:

- 1) absolute values of conditioned iSP area and test iSP area (Fig 7.3A)
- 2) absolute values of conditioned iSP duration and test iSP duration (Fig 7.3B)
- 3) absolute difference between conditioned iSP area and test iSP area (Fig 7.3D)
- 4) absolute difference between conditioned iSP duration and test iSP duration (Fig 7.3E)

The various methods of analysis were also used for cMEP for consistency, as follows:

- 1) absolute values of conditioned cMEP and test cMEP (Fig 7.3C)
- 2) absolute difference between conditioned cMEP and test cMEP (Fig 7.3F)



(Legend to Figure 7.3 is on next page)

[Fig 7.3A] Ipsilateral silent period (iSP) area in the left first dorsal interosseus in response to single or paired transcranial magnetic stimulation to the right primary motor cortex (dashed lines, test iSP area mean \pm s.e.m.; solid line and triangles, conditioned iSP area, respectively); **[B]** iSP duration in response to single or paired transcranial magnetic stimulation in the left first dorsal interosseus (test iSP duration, dashed lines; conditioned iSP duration, solid line and triangles, respectively); **[C]** peak-to-peak MEP amplitude of the right first dorsal interosseus in response to single or paired stimuli (dashed line, mean range of CS1; CS2-CS1, solid line and triangles, respectively) measured simultaneous to [A-B]; **[D]** absolute difference of the MEP amplitude of the right first dorsal interosseus with CS2-CS1 stimulation (conditioned cMEP) and CS1 stimulation (baseline cMEP); **[E]** absolute difference of the iSP duration of CS2-CS1 stimulation (conditioned iSP duration) with iSP duration of CS1 stimulation; **[F]** absolute difference of the MEP amplitude of right first dorsal interosseus with CS2-CS1 stimulation (conditioned cMEP) with the MEP amplitude of CS1 stimulation (baseline cMEP). In all graphs, the abscissa indicates the blocks of interstimulus interval between CS2 and CS1.

From the above figures, it is clear that no matter how the data are expressed, all three measures depend on the ISI between CS2 and CS1. In sum, the area and duration of the iSP as well as the amplitude of the cMEP seem to peak at ISIs 1.5 and 3ms. This was borne out in the statistical analysis summarised in Table 7.1.

Table 7.1A: Two-way ANOVA with "CS MODE" and "ISI"									
	iSP area			iSP duration			cMEP		
	df	F	p	df	F	p	df	F	p
"CS MODE"	1	31.127	<0.001	1	22.691	<0.001	1	17.221	<0.001
"ISI"	8	2.869	0.039	8	2.236	0.089	8	3.048	0.035
"CS MODE" x "ISI"	8	2.685	0.048	8	1.759	0.160	8	7.846	<0.001

Table 7.1B: One-way ANOVA with factor "ISI"			
	conditioned iSP area absolute Δ	conditioned iSP duration absolute Δ	conditioned cMEP absolute Δ
df	8	8	8
F	2.685	1.759	7.846
p	0.048	0.160	<0.001

ISI	Table 7.1C: Post-hoc tests
	conditioned iSP area vs test iSP area
1.3ms	p=0.001
1.5ms	p<0.001
2.0ms	n.s.
2.3ms	n.s.
2.5ms	p=0.006
3.0ms	p=0.001
3.3ms	p=0.024
3.5ms	n.s.
4.3ms	p=0.013

Table 7.1 [A] Two-factorial ANOVA for Experiment 1a with factors “CS MODE” and “ISI” using peak-to-peak MEP amplitude for 11 subjects; **[B]** One-factorial ANOVA for Experiment 1a with factor “ISI” comparing absolute differences of conditioned iSP area, iSP duration or cMEP with baseline test measures for 11 subjects; **[C]** Post-hoc Tukey’s t-test comparing raw values of

conditioned and unconditioned iSP area. n.s. indicates non-significance ($p \geq 0.05$).

A two-factor ANOVA of repeated measures on the raw data (Table 7.1 A-C) with “CS MODE” and “ISI” as main factors revealed a significant interaction between “CS MODE” and “ISI”. Follow up one-factor ANOVAs with “ISI” as main factor showed significant effects for iSP area and cMEP amplitude, indicating that these measures depended on the interval between CS1 and CS2. Post-hoc analysis of the data was conducted to test for intervals where paired CS2-CS1 conditioning produced significant differences to the effect of CS1 alone (Table 7.1C). The results varied depending on the method of analysis, but in general confirmed that maximum effects occurred with ISIs of 1.5 and 3ms. It is not possible to compare the post-hoc effects of cMEP as there was no consensus on how high intensity stimulation of the CTS (~150%AMT) would summate (linearly or non-linearly) but it is well established that whatever the manner of summation, the peaks of facilitatory interaction would occur at 1.5ms and 3.0ms (Tokimura et al 1996; Ziemann et al 1998).

A correlation analysis was performed to test whether absolute differences of conditioned iSP area or conditioned iSP duration correlated with the amplitude of the conditioned cMEP over all ISIs. Only subjects in whom CS2 alone evoked a measurable iSP in every trial were included in the analysis (n=8 subjects out of 13 subjects). The data showed a significant linear correlation for conditioned iSP area (expressed as $iSP_{CS2-CS1} - iSP_{CS1}$) (Fig 7.4A, $r = 0.262$, $p = 0.026$, $n = 72$). Linear correlation for the absolute difference in conditioned iSP duration was not significant (Fig 7.4B, $r = 0.101$, $p = 0.398$, $n = 72$).

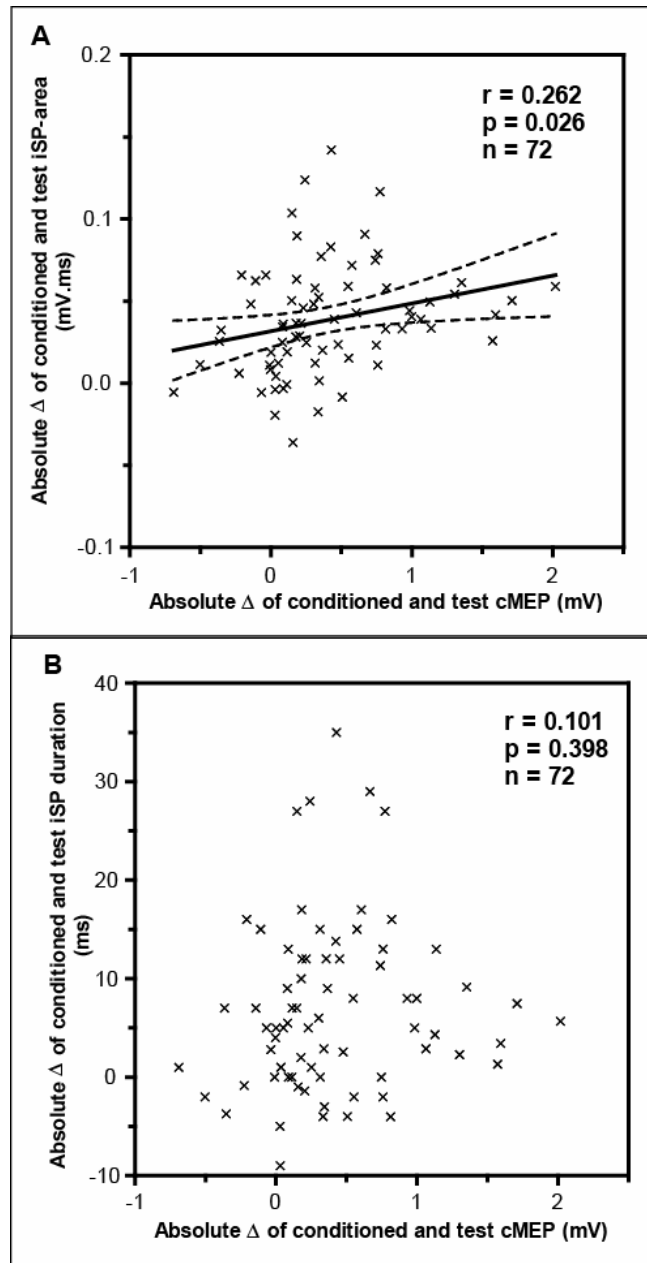
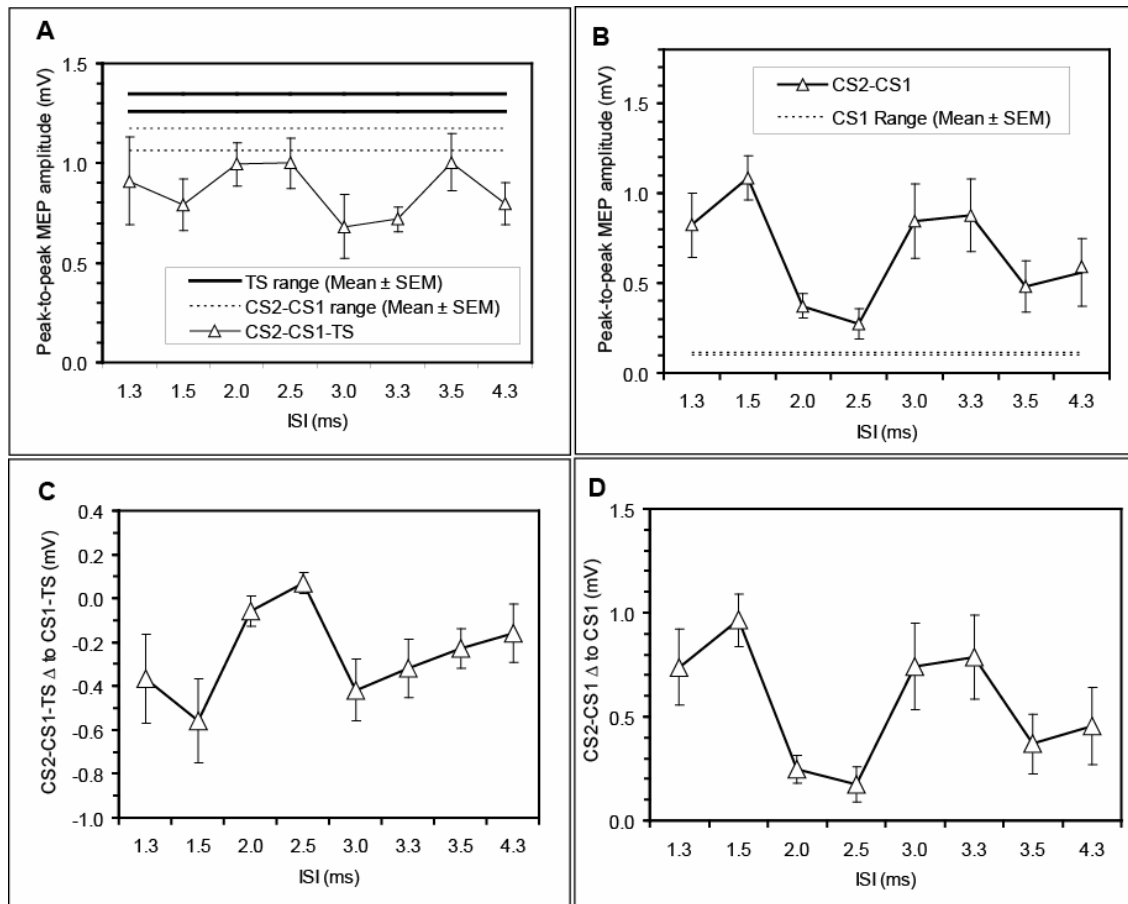


Fig 7.4 Linear correlations of **[A]** conditioned iSP area, or **[B]** conditioned iSP duration with conditioned cMEP represented as absolute difference between conditioned response and test response, for 9 interstimulus intervals (1.3ms, 1.5ms, 2.0ms, 2.3ms, 2.5ms, 3.0ms, 3.3ms, 3.5ms and 4.3ms) of 8 subjects with iSP reliably present in every trace with a single stimulus (13 subjects were tested in total but 5 did not have a reliable iSP). The solid line represents the linear correlation; the dotted lines represent the 95% confidence interval of the correlation.

7.2.3.2 Demonstration of SICF-like effects on IHI

In this experiment, TMS pulses (CS2, CS1) were applied to the right M1 in order to evoke IHI of test MEPs evoked from the left M1. We measured the amplitude of the contralateral MEPs from both hemispheres. Stimulus intensities were adjusted so that the test stimulus (TS) on the left hemisphere evoked a cMEP of about 1mV; the conditioning intensity was adjusted to give a minimal amount of IHI with CS1 alone. The mean of intensity of TS was $116\% \pm 5\%$ RMT and the mean of intensity of CS1 and CS2 was $99\% \pm 6\%$ RMT (or $135\% \pm 10\%$ AMT). The test stimulus alone produced a cMEP amplitude of $1.30\text{mV} \pm 0.13\text{mV}$ which was suppressed by the conditioning stimulus CS1 to $1.12\text{mV} \pm 0.15\text{mV}$ ($85.7\% \pm 9.7\%$) of the test stimulus; paired t-test, $p < 0.01$, $n=9$) (Fig 7.5A).

The principal finding was that the addition of CS2 increased the amount of interhemispheric inhibition over that seen with CS1 alone at specific intervals. Fig 7.5 shows measures of the (transcallosally inhibited) cMEP evoked from the right hemisphere (Fig 7.5A and 7.5C), as well as the cMEP evoked by CS2 and CS1-CS2 from the left hemisphere (Fig 7.5B and 7.5D). At the same intervals as the amount of IHI was maximal, the amplitudes of the cMEPs evoked by CS2-CS1 were largest. The top two graphs (A and B) plot absolute amplitudes of MEP, and the bottom two graphs (C and D) shows the data expressed as differences in the amplitudes of responses with CS1 alone and CS2-CS1 together.



[Fig 7.5A] Peak-to-peak MEP amplitude of the left first dorsal interosseus in response to single, paired or triple stimuli (bold line, mean range of TS \pm s.e.m.; dashed line, mean range of CS1-TS; CS2-CS1-TS, solid line and triangles, respectively); **[B]** Peak-to-peak MEP amplitude of the right first dorsal interosseus in response to single or paired stimuli (dashed line, mean range of CS1; CS2-CS1, solid line and triangles, respectively) measured simultaneous to [A-C]; **[C]** Absolute difference of the MEP amplitude of the left first dorsal interosseus with CS2-CS1-TS stimulation (conditioned IHI) and CS1-TS stimulation (baseline IHI); **[D]** Absolute difference of the MEP amplitude of the right first dorsal interosseus with CS2-CS1 stimulation (conditioned cMEP) and CS1 stimulation (baseline cMEP). In all graphs, the abscissa indicates the interstimulus interval between CS2 and CS1.

Table 7.2 gives the statistical analysis of the results. Two-way repeated measures ANOVA with factors “CS MODE” and “ISI BLOCK” for MEP amplitude showed a significant “CS MODE” X “ISI BLOCK” interaction (Table 7.2A, $p < 0.05$). Post hoc one-way ANOVAs with “ISI BLOCK” as main factor (Table 7.2B) confirmed that no matter which way the data were analysed, the amount of IHI as well as the amplitude of the cMEP elicited from the right (CS2-CS1) hemisphere varied with ISI. Paired comparisons to detect at what interval the largest effects of ISI on IHI was at ISIs of about 1.5 and 3ms (Table 7.2C). Post-hoc tests of ISI on cMEP were not performed as there was no consensus on how high intensity stimulation of the CTS (~135%AMT) would summate (linearly or non-linearly) but it is well established that whatever the manner of summation, the peaks of facilitatory interaction would occur at 1.5ms and 3.0ms (Tokimura et al 1996; Ziemann et al 1998).

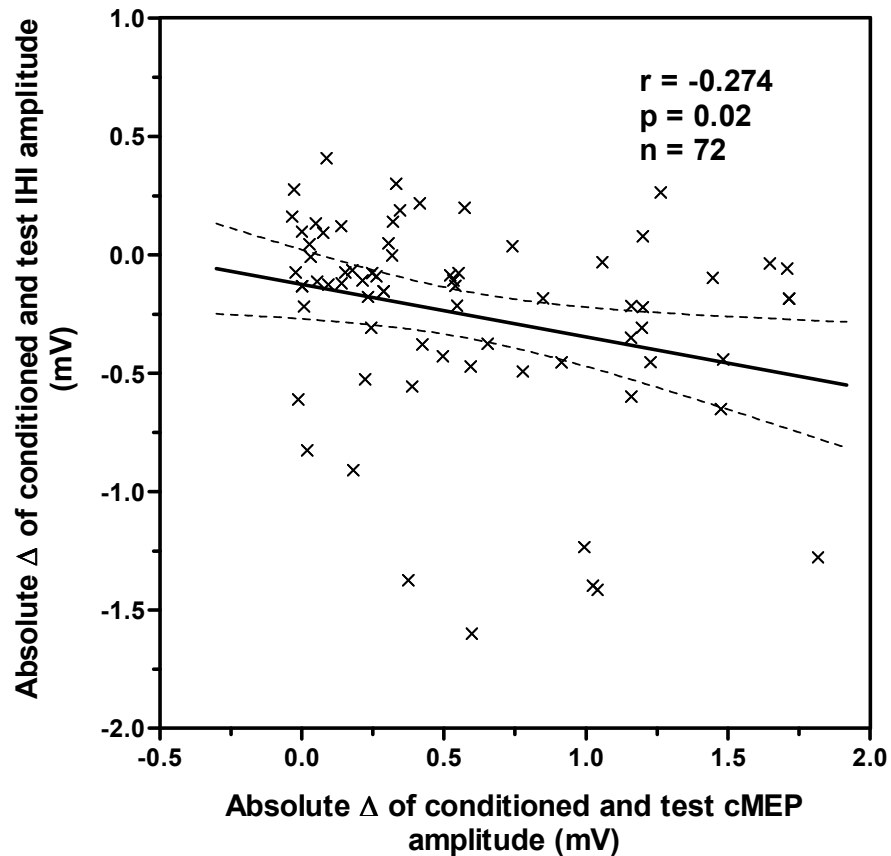
Table 7.2A: Two-way ANOVA with "CS MODE" and "ISI"						
	IHI			cMEP		
	df	F	p	df	F	p
"CS MODE"	1	14.316	0.005	1	34.120	<0.001
"ISI"	7	0.689	0.680	7	3.024	0.009
"CS MODE" x "ISI"	7	2.625	0.020	7	4.511	<0.001

Table 7.2B: One-way ANOVA with factor "ISI"		
	cond-IHI Absolute Δ to baseline IHI	cond-cMEP Absolute Δ to 1x baseline cMEP
df	7	7
F	2.625	4.511
p	0.020	<0.001

ISI	Table 7.2C: Post-hoc tests
	1x cond-IHI vs. 1x baseline-IHI
1.3ms	n.s.
1.5ms	p=0.019
2.0ms	n.s.
2.5ms	n.s.
3.0ms	p=0.018
3.3ms	p=0.043
3.5ms	p=0.037
4.3ms	n.s.

[**Table 7.2A**] Two-factorial ANOVA for Experiment 1b with factors "CS MODE" and "ISI" using peak-to-peak MEP amplitude of baseline IHI and conditioned IHI or baseline cMEP and conditioned cMEP for 9 subjects [**B**] One-factorial ANOVA for Experiment 1b with factor "ISI" comparing conditioned IHI or cMEP and baseline IHI and cMEP for 9 subjects; [**C**] Post-hoc Tukey's t-test comparing peak-to-peak MEP amplitudes of conditioned IHI to baseline IHI. n.s. indicates non-significance ($p>0.05$).

As with the iSP, we next asked whether ISI affected IHI and cMEP in the same way. The conditioned IHI (CS2-CS1-TS) and the conditioned cMEP (CS2-CS1) showed a significant negative correlation across the eight interstimulus intervals in all subjects regardless of method for data analysis (Fig 7.6, $p < 0.05$, $n = 72$).



[Figure 7.6] Linear correlations of conditioned IHI amplitude with conditioned cMEP amplitude represented as absolute difference measures respectively for 8 interstimulus intervals (1.3ms, 1.5ms, 2.0ms, 2.5ms, 3.0ms, 3.3ms, 3.5ms and 4.3ms) of all 9 subjects. The solid line represents the linear correlation; the dotted lines represent the 95% confidence interval of the correlation.

7.2.4 Discussion

TMS of the motor cortex evokes activity in the corticospinal system that is detected by measuring the amplitude of MEPs. The excitability of this system is controlled by a number of intrinsic cortical circuits that have been explored with paired pulse TMS methods. Of these, the best described are short interval intracortical inhibition (SICI) and short interval intracortical facilitation (SICF) that are thought to be due to activity in local GABA_A inhibitory interneurons and repetitive facilitatory I-wave input respectively.

In addition to activation of the corticospinal output, motor cortex stimulation can evoke activity in transcallosal projections. These produce a silent period in the EMG of contracting ipsilateral muscles (iSP) as well as interhemispheric inhibition (IHI) of MEPs evoked from the opposite motor cortex. Recently Trompetto et al (2004) showed that transcallosal outputs from motor cortex receive inhibitory input analogous to corticospinal SICI. We refer to this as SICI_{iSP} to distinguish it from the usual corticospinal SICI (SICI_{cMEP}). The present experiments extend these similarities between corticospinal and transcallosal systems by showing that transcallosal outputs have SICF-like properties. We suggest that the data are compatible with a model in which the transcallosally projecting pyramidal neurones of cortical layer III are controlled by circuits similar to those that control layer V pyramidal neurones of the corticospinal tract. However, it should be noted that there are multiple interhemispheric effects. We only tested IHI at an ISI of 40ms; IHI at short intervals (8-12ms) may have a different mechanism (Chen et al 2003). In addition, very low intensity conditioning pulses evoke a weak interhemispheric facilitation that has another mechanism (Ugawa et al 1993; Hanajima et al 2001). No firm conclusions can be made about interneuronal control of these other pathways.

7.4.1 Site of facilitatory interaction

We investigated transcallosal output with two methods: the ipsilateral silent period (iSP), and paired pulse interhemispheric inhibition (IHI). As noted in the Introduction, there is good evidence that both effects are mediated predominantly by pathways running through the corpus callosum, presumably in the transcallosal axons of layer III pyramidal neurones (Jacobsen et al 1974; Jones et al 1979).

Experiments 1a and 1b showed that if the intensity of the conditioning stimulus was reduced to threshold levels for iSP or IHI, pairs of stimuli at intervals of around 1.5 and 3.0 ms consistently produced much greater transcallosal inhibition than for other intervals. Since this time course is exactly the same as SICF on cMEP (Tokimura et al 1996; Ziemann et al 1998), the most parsimonious explanation is that a similar mechanism is involved.

As it is unknown if IHI and iSP summates linearly or nonlinearly, post-hoc tests were done on absolute data. As such, cautious interpretation of post-hoc tests is necessary. However whatever the method of analysis, it is clear that the interstimulus intervals where post-hoc differences are consistently largest are interstimulus intervals of 1.5ms and 3.0ms; conversely, whatever the method of analysis, the interstimulus intervals where there were no post-hoc differences in iSP area, iSP duration and IHI were 2.0ms.

Nevertheless whatever the method of data analysis for iSP and IHI, the interstimulus intervals where post-hoc differences are consistently present in all cases are interstimulus intervals of 1.5ms and 3.0ms. The relationship between SICF in iSP and SICF in IHI with SICF in cMEP are also confirmed by correlation analysis indicating the presence of a positive linear correlation for iSP area with cMEP amplitude and a negative linear correlation of IHI with cMEP amplitude. iSP duration was not correlated with cMEP amplitude, and this probably reflects that duration of inhibition is not equivalent to degree of inhibition.

The question arises as to the location of the paired pulse interaction on iSP/IHI. This could occur either within the stimulated hemisphere, or, since each individual pulse was strong enough to activate a threshold transcallosal effect, it could also have occurred in the opposite hemisphere, or even in the spinal cord. In the absence of direct recordings of activity at each site it is difficult to be certain. However, transcallosal axons are smaller diameter than the larger corticospinal axons, and therefore will have a longer refractory period after firing an action potential. Direct measurements show that the absolute refractory period of corticospinal axons is about 1ms, with the relative refractory period lasting for at least a further 2ms (Deletis et al 2001; Novak et al 2004). This means that at the first peak of paired pulse facilitation of iSP/IHI (1.3-1.5ms), transcallosal axons are likely to be still refractory from the first TMS pulse, and therefore difficult to recruit by a second identical stimulus. Thus we conclude that at the least the first peak of interaction at 1.5ms is likely to have occurred within the stimulated hemisphere rather than at any other site.

7.4.2. Nature of facilitatory interaction

I-waves are believed to result from rhythmic excitatory trans-synaptic input of corticospinal neurons (Amassian & Cracco 1987; Ziemann & Rothwell 2000) the timing of which may be due to properties of the neurons, synapses or the interneuron network stimulated. Our experiment indicates that excitatory input onto transcallosal neurons also has the same rhythmic timing as I-waves on corticospinal neurons. This suggests that both these networks of excitatory interneurons have similar properties. It is also worthwhile to note that in a recent paper (Koch et al. 2006), the premotor cortex appears to have a different SICF-like rhythmicity (0.8ms rather than 1.5ms) which suggests that this rhythmicity is specific to the primary motor cortex.

The similar rhythmicity of I-wave interaction may suggest that the corticospinal and transcallosal neurons share a common excitatory interneuron network. On the other hand, it may also suggest that I-waves are a shared network property of the excitatory interneuronal networks rather than a shared network of neurons. It is not possible to exclude either of these possibilities based on our study.

It is interesting to note the low level of correlation between the corticospinal SICF and the transcallosal SICF ($r^2 = 0.069-0.075$ depending on the measure used for transcallosal inhibition). It could be extrapolated from this that the intracortical circuits responsible for SICF in TC neurons and CTS neurons are only minimally overlapping as mentioned above; however it could also suggest that SICF (via transcallosal inhibition) has only a minor inhibitory effect on contralateral corticospinal neuronal excitability. Certainly a recent study in the rat has also shown that individual deep cortical layer pyramidal cells both receive and send direct inputs to callosal fibres monosynaptically (Karayannis et al., 2007) suggesting the latter explanation, but this is unproven.

7.3 Effect of rTMS on transcallosal circuits

As intracortical circuits that synapse onto transcallosal neurons show SICF-like effects and SICI-like effects (Trompetto et al., 2004), it is likely they behave in a similar manner to intracortical circuits that synapse onto corticospinal neurons. It has been demonstrated that intracortical circuits that synapse onto corticospinal neurons can be modulated by repetitive transcranial magnetic stimulation (rTMS) (Peinmann et al., Quartarone et al., 2005), but it is unknown if the intracortical circuits that modulate transcallosal neurons demonstrate plasticity in response to rTMS. This is the aim of the next study.

7.3.1 Study design

The study design of experiment 2 on the effect of rTMS on corticospinal intracortical inhibition and transcallosal inhibition is summarised as follows (Fig 7.7)

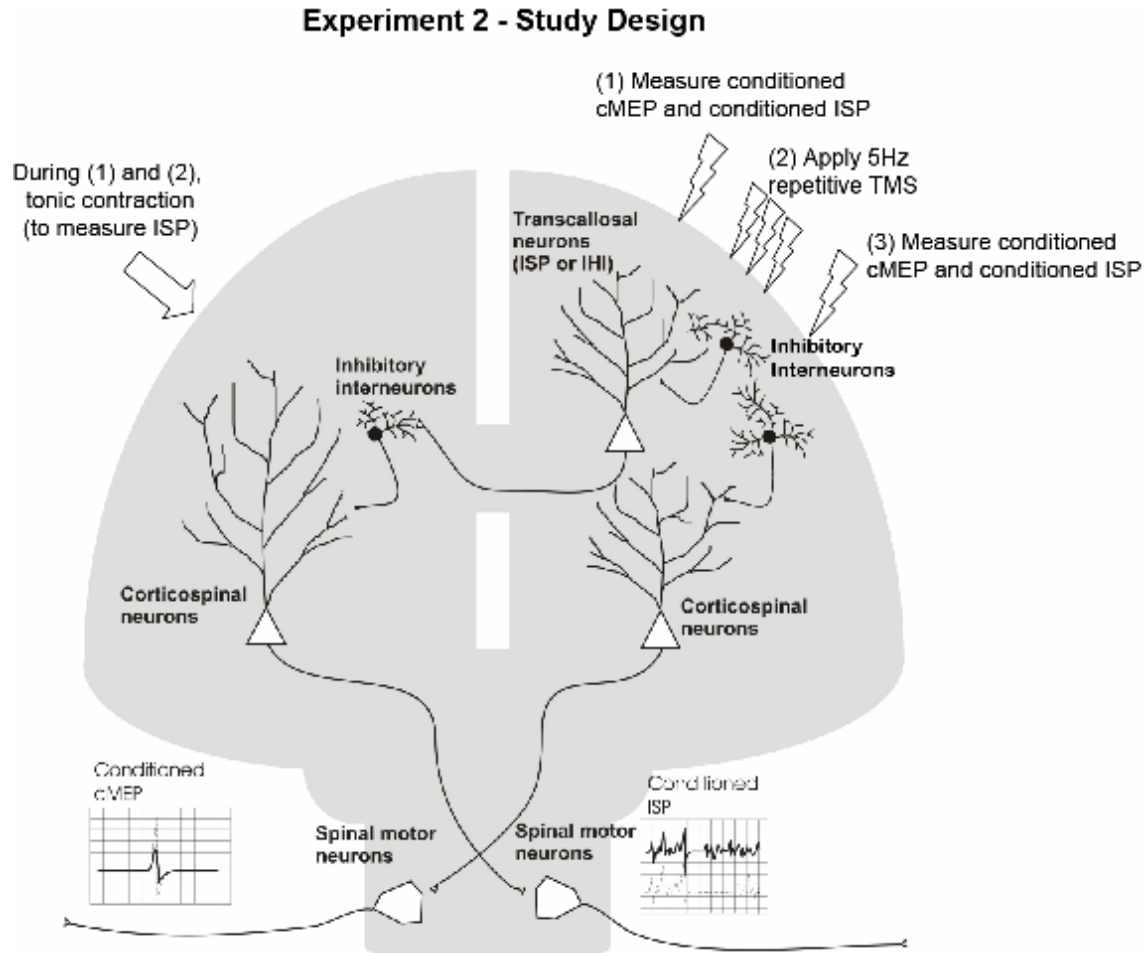


Fig 7.7. Study design of experiment 2 to study the effect of rTMS on the inhibitory intracortical circuits synapsing onto corticospinal and transcallosal output neurons.

8 right handed subjects (5 males and 3 females, mean age 30.0 ± 3 years, range 25-35) were recruited and gave their written informed consent. A repeated-measures study design was used with measurement blocks being conducted before and immediately after a session of rTMS. Short-interval intracortical inhibition was measured using a paired-pulse paradigm (Kujirai et al. 1993; Trompetto et al. 2004). The test stimulus was set at an intensity between

150%-200% AMT where there was a clear iSP recognisable in every trial. In order to evaluate the threshold of inhibition for both iSP and cMEP various intensities of conditioning stimulus were measured (70%, 90%, 110%, 130% AMT), starting from 70%AMT that is known to be at or below threshold for inducing SICI (Kujirai et al 1993; Trompetto et al 2004)

Four blocks (one for every CS intensity) of 30 trials were performed consisting of three randomly intermixed conditions (10 trials for each condition): TS given alone and TS preceded by CS at two different intervals (2 ms and 3 ms). The four stimulation conditions of different CS intensities were tested in a pseudo-randomised order. iSP and cMEP measurements were carried out in an identical fashion to experiment 1a with contraction of the left FDI at approximately 50% of maximum force while maintaining the corresponding right muscle at rest. The test stimulus intensity was kept constant after rTMS for each subject as was the order of the testing of the conditioning stimuli intensity.

rTMS was applied over the left M1 with a Magstim Rapid stimulator and a flat figure-of-eight coil with mean loop diameter of 9 cm. The magnetic stimulus had a biphasic waveform with a pulse width of approximately 300 μ s. We applied two conditioning trains of 5 Hz rTMS (600 stimuli in total). The intensity of rTMS was set at 90% RMT. We chose this protocol as Quartarone et al. (2005) demonstrated that it can selectively shape the excitability of the intracortical circuits without significantly affecting corticospinal excitability.

7.3.2 Data analysis

The measurement of iSP and cMEP was identical to experiment 1a. However, in experiment 2, as the inhibition of iSP was being measured (rather than a summation of iSP), we normalised the conditioned iSP against the test iSP and expressed the result as a percentage:

$$\text{Conditioned iSP (as \% of test)} = \frac{[\text{iSP area in conditioned response}]}{[\text{iSP area in test response}]}$$

For statistical analysis, two-tailed Student's paired t-test was used to compare the single-pulse measures (the motor thresholds, iSP, the duration of the iSP and the amplitude of the cMEP) in the test responses before and after the rTMS.

The effect of the paired pulse stimulation before rTMS was analysed using a two-factorial analysis of variance (ANOVA) for repeated-measures using the factors "INTENSITY" (70%, 90%, 110%, 130% AMT) and the "MODE OF STIMULATION" (single test-pulse, paired-pulse with 3ms ISI and paired-pulse with 2ms ISI). The analysis was performed with the raw data of iSP area and cMEP obtained before the rTMS. If ANOVA showed a significant effect, we performed post-hoc comparisons using the Tukey least significant difference (LSD) test to compare directly the experimental conditions. This was to determine which intensities of conditioning stimulation produced significant effects on SICI_{iSP} and $\text{SICI}_{\text{cMEP}}$ compared with baseline.

To analyse the effect of rTMS on paired-pulse stimulation, only intensities where there was SICI_{iSP} and $\text{SICI}_{\text{cMEP}}$ at baseline (from above) were analysed using the normalised iSP and cMEP. A three-factorial ANOVA for repeated-measures was performed using the factors "INTENSITY" (intensities where there was significant SICI_{iSP} or $\text{SICI}_{\text{cMEP}}$ at baseline), "TIME" (before rTMS, after rTMS) and "ISI" (2ms ISI, 3ms ISI).

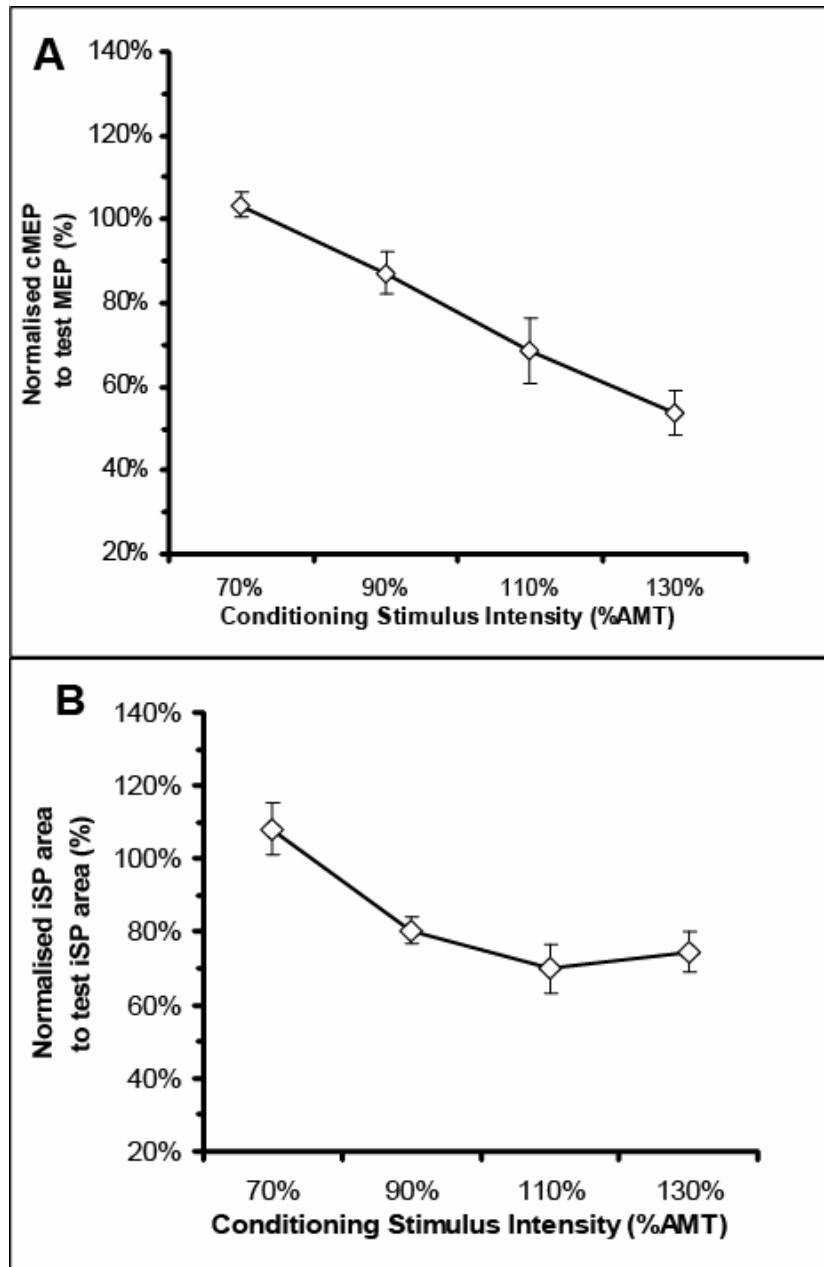
For all ANOVAs, the Greenhouse-Geisser method was used if necessary to correct for non-sphericity and post-hoc Tukey (LSD) tests were done for significant results to compare directly the experimental conditions.

7.3.3 Results

None of the participants reported any adverse effect during the course of the study.

7.3.3.1 SICI_{cMEP} and SICI_{iSP}

In the “classical” SICI paradigm of Kujirai et al (1993), a subthreshold conditioning stimulus (CS) is used to suppress the cMEP evoked 2-3 ms later by a suprathreshold test stimulus (TS); we refer to this as SICI_{cMEP}. Trompetto et al. (2004) recently showed that the CS also had a similar effect in reducing the area of the iSP evoked in the ipsilateral contracting muscle by the TS (SICI_{iSP}). We reproduced this finding at baseline pre-rTMS (Fig 7.7, Table 7.3) showing that SICI_{cMEP} and SICI_{iSP} occur at 90% to 130% AMT but not at 70% AMT (Fig 7.7, Table 7.3A-B). The two-factorial repeated measures ANOVA for “CONDITIONING INTENSITY BLOCK” and “MODE OF STIMULATION” and the post-hoc analysis (Table 7.3A-B) clearly indicate that the threshold for inducing SICI_{cMEP} and SICI_{iSP} during contraction was 90%AMT and above. This lack of conditioning effect at 70%AMT is similar to the original finding where SICI_{cMEP} and SICI_{iSP} were only reliably found at 80%RMT (Trompetto et al. 2004)



[Fig 7.8A-B] Effect of the conditioning stimulus on the cMEP (A) and iSP area (B) before and after the rTMS averaged for 2ms and 3ms ISI. *Abscissa* indicates the CS intensity expressed as a percentage of the active motor threshold. *Ordinate* indicates the size of the conditioned response, expressed as a percentage of the unconditioned test response.

Table 7.4A: Two-factorial ANOVA of SICI_{cMEP} and SICI_{iSP} before rTMS				
Factor(s)	Absolute cMEP		Absolute iSP area	
	F	p	F	p
“CONDITIONING INTENSITY BLOCK”	0.6	0.618	0.3	0.852
“MODE OF STIMULATION”	10.2	0.011*	12.3	0.001*
“CONDITIONING INTENSITY BLOCK” x “MODE OF STIMULATION”	7.5	<0.001*	3.2	0.01*

Table 7.4B: Post-hoc analysis			
Factor	Comparison	cMEP	iSP area
“Mode of Stimulation”	Test pulse only vs 2ms paired-pulse	0.004*	0.007*
	Test pulse only vs 3ms paired-pulse	0.018*	0.002*
“Conditioning intensity block” x “Mode of Stimulation”	70%AMT (2ms paired pulse vs test pulse only)	0.399	0.067
	70%AMT (3ms paired pulse vs test pulse only)	0.528	0.943
	90%AMT (2ms paired pulse vs test pulse only)	0.008*	0.007*
	90%AMT (3ms paired pulse vs test pulse only)	0.047*	0.005*
	110%AMT (2ms paired pulse vs test pulse only)	0.027*	0.023*
	110%AMT (3ms paired pulse vs test pulse only)	0.032*	0.030*
	130%AMT (2ms paired pulse vs test pulse only)	0.005*	0.044*
	130%AMT (3ms paired pulse vs test pulse only)	0.013*	0.010*

[Table 7.3A] Two factorial ANOVA with repeated measures of absolute cMEP

amplitude or absolute iSP area before rTMS using the following factors:

“CONDITIONING INTENSITY BLOCK” (70% AMT, 90% AMT, 110% AMT, 130%AMT) and “MODE OF STIMULATION” (test pulse only, 2ms paired-pulse, 3ms paired-pulse) for 8 subjects. **[B]** Post-hoc analysis of the

significant interactions indicating that the conditioning intensity 70% AMT was significantly different from 90%-130% AMT and was not significantly different from test-pulse alone. For both tables, * indicates significant effect of at least $p < 0.05$.

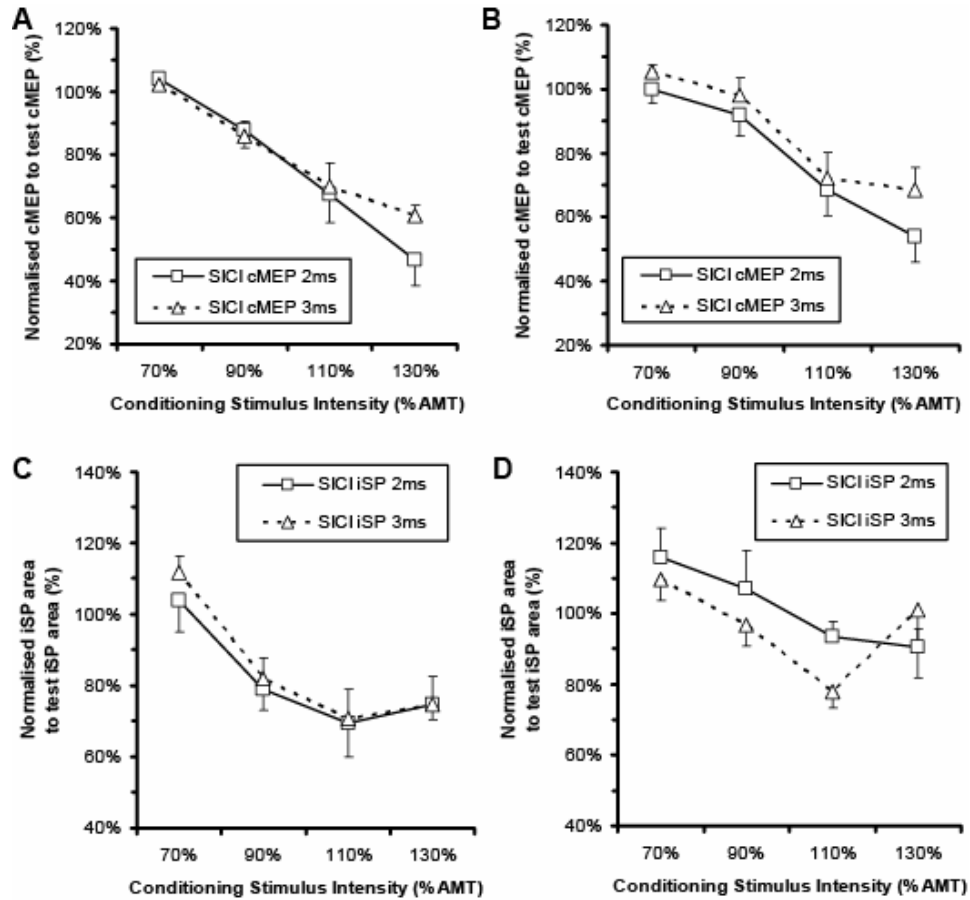
7.3.3.2 Effect of rTMS on $SICI_{cMEP}$ and $SICI_{iSP}$

Here we ask whether these two effects are influenced in the same way by preconditioning M1 with rTMS at 5 Hz. rTMS was applied at an intensity of 90% RMT, which was equal to $39\% \pm 5\%$ maximal stimulator output (MSO). The TS intensity was set to evoke a cMEP of approximately 1mV; this intensity was the same before and after rTMS ($63\% \pm 6.8\%$ MSO before and $62\% \pm 7.7\%$ MSO after). Pre-conditioning with rTMS decreased the area of the iSP evoked by TS alone ($p < 0.01$) but had no effect on the amplitude of the cMEP (Table 7.4).

Parameter	Before rTMS	After rTMS	t-test
RMT (% of maximal stimulator output)	33.1 ± 6.1	33.8 ± 6.2	n.s.
AMT (% of maximal stimulator output)	25.3 ± 4.9	25.0 ± 4.6	n.s.
Mean cMEP amplitude during ipsilateral contraction (mV)	3.1 ± 1.9	3.2 ± 1.9	n.s.
Mean iSP area (mv.ms)	0.16 ± 0.07	0.14 ± 0.06	$p < 0.01$
iSP duration (ms)	33.5 ± 9.2	30.5 ± 10.4	n.s.

[Table 7.4] Active and resting motor threshold, resting MEP amplitudes, resting iSP area and duration of the iSP before and after 600 stimuli of subthreshold 5Hz rTMS to the left M1. RMT: resting motor threshold; AMT: active motor threshold; n.s.: not significant; each value corresponds to the mean (\pm SEM) of nine subjects.

The next question was whether 5 Hz rTMS at 90%RMT affected $SICI_{cMEP}$ and $SICI_{iSP}$. As there was no $SICI_{cMEP}$ and $SICI_{iSP}$ at 70%AMT (Table 7.3A-B), 70%AMT was excluded from further analysis. 600 stimuli of 5 Hz rTMS at 90% RMT decreased $SICI_{cMEP}$ (Fig 7.8, Table 7.5) This was confirmed by a significant effect of “TIME” ($F(1) = 6.84$, $p=0.035$) in a 3-factor ANOVA with “TIME”, “INTENSITY” and “ISI” as factors. rTMS also decreased $SICI_{iSP}$ (Fig 7.8, Table 7.5) with a main effect of “TIME” ($F = 10.71$, $p=0.014$).



[Fig 7.9] Effect of varying ISI of the conditioning stimulus on the **[A]** cMEP before rTMS, **[B]** cMEP after rTMS, **[C]** iSP area before rTMS and **[D]** iSP area after rTMS. *Abscissa* indicates the CS intensity expressed as a percentage of the active motor threshold. *Ordinate* indicates the size of the conditioned response, expressed as a percentage of the unconditioned test response. (solid line and squares, $SICI_{2ms}$, dotted line and triangles, $SICI_{3ms}$).

Table 7.5: Three-factorial ANOVA of SICI_{cMEP} and SICI_{iSP} before and after rTMS				
Factor(s)	cMEP		iSP area	
	F	p	F	p
“TIME”	6.81	0.035*	10.72	0.014*
“INTENSITY”	24.78	<0.001*	1.89	0.187
“ISI”	4.39	0.074	0.11	0.752
“TIME” x “INTENSITY”	0.32	0.730	0.10	0.908
“TIME” x “ISI”	0.83	0.391	3.08	0.122
“ISI” x “INTENSITY”	3.39	0.630	1.25	0.316
“TIME” x “INTENSITY” x “ISI”	1.10	0.360	0.86	0.446

[Table 7.5] Three factorial ANOVA with repeated measures using the following factors: “TIME” (Pre-rTMS, post-rTMS); “INTENSITY” (90% AMT, 110% AMT, 130%AMT) and “ISI” (2ms paired-pulse, 3ms paired-pulse) for 8 subjects. 70%AMT conditioning intensity was excluded from the analysis as Table 4 shows that there are no SICI effects at that intensity. * indicates significant effect of at least $p < 0.05$.

7.3.4 Discussion

5 Hz rTMS reduced SICI_{cMEP} whilst having no effect on the amplitude of corticospinal cMEPs evoked by a single pulse TMS as reported previously (Di Lazzaro et al 2002; Quartarone et al 2005). There were two findings in the present experiment. The first confirmed previous observations of Trompetto et al (2004) that SICI_{cMEP} and SICI_{iSP} were measurable during ipsilateral muscle activation with conditioning intensity of 90%AMT and above, and SICI_{cMEP} and SICI_{iSP} both increased with increasing intensity of the conditioning

stimulus over a range from 90-130% AMT (Table 7.4A-B). It may seem surprising that $SICI_{cMEP}$ could be elicited in view of the ongoing contraction of the opposite FDI that was required for measurement of the iSP. Muellbacher et al (2000) had reported that SICI was greatly depressed in such conditions. However, as in experiment 1, the intensity of the test pulse had been adjusted to evoke a clear iSP. This meant that the contralateral MEP was larger than the usual 1mV peak-to-peak conventionally used to evaluate $SICI_{cMEP}$ (Kujirai et al. 1993, Fisher et al. 2002, Chen 2004). Larger MEPs are associated with increased $SICI_{cMEP}$ (Sanger et al. 2001), therefore our experimental protocol may favour demonstrating $SICI_{cMEP}$. It is also interesting to note that $SICI_{iSP}$ (but not $SICI_{cMEP}$) appears to saturate at about 130%AMT (Fig 7.8A, Fig 7.8C and Table 7.6). This is difficult to interpret; it may indicate that interneurons of $SICI_{cMEP}$ and interneurons of $SICI_{iSP}$ are distinct, but it is important to stress that at such high intensities, the conditioning stimulus is suprathreshold for producing both corticospinal volleys and SICF-like phenomena, which complicate the interpretation. The second finding was that 5 Hz rTMS reduced $SICI_{iSP}$ in the same way as it did $SICI_{cMEP}$. We note that although $SICI_{cMEP}$ was less effective after 5Hz rTMS, it was not completely abolished as reported by Quartarone et al (2005). One possible reason for the discrepancy is that we found preserved $SICI_{cMEP}$ after rTMS only when we used relatively high conditioning pulse intensities (110% and 130%AMT) which were not tested in the previous studies. $SICI_{iSP}$ was reduced by 5Hz rTMS in parallel with $SICI_{cMEP}$ (Table 7.2). Furthermore the effect was the same at all intensities of the conditioning stimulus. This implies that the inhibitory interneurons that synapse onto transcallosal neurons are regulated in a similar manner by rTMS as the inhibitory interneurons that synapse onto the corticospinal cells. There was one difference between the effect of 5Hz rTMS on cMEP and iSP: it had no effect on cMEP, whereas there was a small reduction in the depth of iSP. This effect was unexpected as we had anticipated that high-frequency rTMS would, if anything, increase

(rather than decrease) the excitability of transcallosal neurons (Cincotta et al 2005). One possible explanation may relate to the fact that the transcallosal effects between the hemispheres are not purely inhibitory; there is also a low threshold facilitatory pathway that is more difficult to study and whose effect is usually masked by the higher threshold and stronger inhibition (Ugawa et al 1993; Hanajima et al 2001). 5Hz rTMS at 90%RMT may have preferentially affected the lower threshold transcallosal facilitatory neurons, increasing their excitability thus producing an apparent reduction in iSP. Whatever the case, the effect of 5Hz rTMS on iSP is small.

7.4 Conclusion

In summary, this data demonstrate that the control of transcallosal connections between the two hand areas of motor cortex is very similar to that described for the corticospinal outputs from the same area of cortex. Thus, there is evidence for I-wave facilitatory interaction of transcallosal projections as well as short interval inhibitory inputs explored by SICI.

Additionally, the population of SICI-like interneurons that control the transcallosal neurons appear in many ways to behave similarly to those that control corticospinal neurons after subthreshold 5 Hz rTMS. We conclude that intracortical circuits which synapse onto transcallosal and corticospinal neurons in layer III and V of the cortex have similar network properties, and that this may be relevant for effective control of bilateral hand movement.

Chapter 8

Theta burst stimulation and sequence learning

Work described in this chapter have published:

Wilkinson L, Teo JT, Obeso I, Rothwell JC, Jahanshahi M. The contribution of the primary motor cortex is essential for probabilistic implicit sequence learning:

evidence from theta burst magnetic stimulation. J Cogn Neurosci. 2009 Mar 20.

[Epub ahead of print]

8.1 Introduction

It has been suggested that implicit (unconscious) and explicit (conscious) memory are separable learning systems e.g. (Squire & Zola, 1996). The implicit system is believed to be involved in motor skill learning acquired incidentally with practice (e.g. riding a bicycle, playing golf), whereas the explicit system is considered to play a role in the acquisition of knowledge in a more intentional way (e.g. remembering lists of words). Furthermore, it has been proposed that the striatal structures with their cortical projections support implicit learning whereas the cortico-limbic-diencephalic structures are the substrate for explicit (conscious) learning e.g. (Cohen & Squire, 1980).

One paradigm that has been developed to study implicit learning in the laboratory is the serial reaction time (SRT) task (Nissen & Bullemer, 1987). Typically, on each trial of the SRT task a target appears in one of four locations and participants must respond as quickly as possible by pressing a corresponding key on a keypad, participants perform several blocks of trials (e.g. 10 blocks of 100 trials) and reaction times (RTs) are measured. Unknown to participants, the majority of targets actually appear in a pre-determined repeating sequence of box locations (e.g. 3-4-2-3-1-2-1-4-3-2-4-1). The sequence can be presented in either a deterministic or a probabilistic way. If mean RTs across blocks become faster for the sequence relative to the random or pseudo-random trials then it can be inferred that participants learned the trained sequence.

Imaging studies have revealed the functional anatomy of implicit motor sequence learning and have shown that such learning is associated with activation of the primary motor cortex supplementary motor area (SMA) and premotor cortex, dorsolateral prefrontal cortex (DLPFC) and the putamen and caudate e.g. (Grafton et

al., 1995; Hazeltine et al., 1997; Poldrack et al., 2005; Schendan et al., 2003; Seidler et al., 2005). However, functional imaging does not reveal whether the contribution of these various brain regions to implicit motor sequence learning is essential or not. To address this question the technique of repetitive transcranial magnetic stimulation (rTMS) to induce ‘virtual lesions’ has been used in several studies. Early studies have suggested that deterministic SRT learning was impaired by rTMS over the DLPFC but not by rTMS over the SMA (Pascual-Leone et al., 1996) or the primary motor cortex (M1) (Pascual-Leone et al., 1999). However, more recent studies on the effect of stimulation of the primary motor cortex contradict this result. Motor sequence learning was enhanced by 5Hz rTMS over the primary motor cortex (Kim et al., 2004), whereas both anodal and cathodal transcranial direct current stimulation (atDCS, ctDCS) over this area enhanced learning if they were delivered during the task (Nitsche et al., 2003) and they impaired learning if delivered prior to learning (Kuo et al., 2008).

Similarly, while rTMS over the SMA was previously shown not to affect SRT learning (Pascual-Leone et al., 1996), it affected transfer of knowledge to the non-performing hand (Perez et al., 2007). The role of DLPFC in sequence learning also appears to be more complex. Although rTMS over the DLPFC delivered during a deterministic SRT task (Pascual-Leone et al., 1999; Pascual-Leone et al., 1996) impaired normal learning, this impairment disappeared if there was no spatial component to the visual cues (Robertson et al., 2001). Furthermore, an impairment of SRT learning in the left hand of a patient with a focal lesion of the left cerebellum was shown to be restored by rTMS over both the cerebellum and DLPFC delivered prior to SRT learning (Torriero et al., 2007).

This inconsistent pattern of results partly relates to the different methodologies of delivering brain stimulation. As mentioned previously, some experimenters delivered stimulation during the SRT task (Nitsche et al., 2003; Pascual-Leone et al., 1999; Pascual-Leone et al., 1996) or interspersed with the SRT task (Kim et al., 2004). The delivery of stimulation during performance of the SRT task is compounded by problems of distraction as rTMS produces a palpable scalp sensation and a loud ‘click’, which may interfere with task performance and learning. Finally, all of the above studies used a deterministic SRT task which is a less sensitive index of learning and less likely to foster learning that is truly explicit compared to the probabilistic SRT task which provides an ‘on line’ index of learning on every block and the element of noise in the probabilistic sequence blocks explicit knowledge and promotes implicit learning of the sequence.

Theta burst rTMS (TBS) is a more recent rTMS technique (Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005) which can be delivered relatively rapidly (< 3 minutes) and has been shown to produce excitatory (intermittent TBS) or inhibitory (continuous TBS) effects on cortical excitability lasting for 40 minutes after the stimulation has ended. This longer lasting stimulation effect allows an ‘offline’ approach with the participant being able to perform the SRT task undistracted by concurrent stimulation and unconstrained by the stimulating coil. TBS has been shown to produce plastic changes in human motor cortex (Huang et al., 2005), but to date the effects of TBS on learning have not been investigated. In this study we had two objectives. Our first aim was to assess whether the contribution of the M1, SMA and DLPFC to implicit sequence learning are essential by applying continuous inhibitory TBS over these areas immediately before performance of a probabilistic SRT task. We predicted that if the contribution of these areas to learning is essential, then inhibitory TBS over the

area would impair subsequent implicit sequence learning. Our second aim was to assess whether implicit sequence learning could be improved by intermittent excitatory TBS over M1.

8.2 Methods

8.2.1 Participants

40 right-handed healthy volunteers were recruited, all of whom met the safety criteria for transcranial magnetic stimulation (Keel, Smith, & Wassermann, 2001). None of the participants had any neurological disorder or history of psychiatric illness, drug or alcohol abuse or were on any drug treatments that might influence performance. The study was approved by the Joint Ethics Committee of the Institute of Neurology and The National Hospital for Neurology and Neurosurgery. Informed consent was obtained from all participants. Participants were randomly assigned either to:

- a) Sham stimulation group (n = 8, 4 female) aged 22-36 years (M = 27.63, SD = 4.44).
- b) Continuous (Inhibitory) TBS over M1 group (n = 8, 5 female) aged 24-37 years (M = 30.63, SD = 4.57).
- c) Continuous (Inhibitory) TBS over SMA group (n = 8, 4 female) aged 20-33 years (M = 25.38, SD = 4.78).
- d) Continuous (Inhibitory) TBS over DLPFC group (n = 8, 5 female) aged 24-38 years (M = 30.38, SD = 5.48).
- e) Intermittent (Excitatory) TBS over M1 group (n = 8, 6 female) aged 22-36 years (M = 27.63, SD = 4.44).

8.2.2 Serial Reaction Time (SRT) task

In the deterministic SRT task (employed in the majority of SRT studies) this sequence is repeatedly presented during all blocks with the exception of a single 'transfer' block (e.g. block 9) in which different random or pseudo-random trials are introduced. It is often concluded that knowledge acquired during the deterministic SRT task is implicit or unconscious however, in some studies participants have been shown to develop conscious sequence knowledge during supposedly implicit SRT learning e.g. (Wilkinson & Shanks, 2004). To address this concern, experimenters have attempted to minimize the chance that SRT learning will be explicit by adopting probabilistic, rather than deterministic, sequence presentation.

In the probabilistic SRT task (employed here) the sequence is presented such that on any single trial there is an 85% chance that the target will appear according to the sequence and a 15% chance that it will appear according to an alternate sequence. Hence, the element of noise during the probabilistic sequence presentation reduces the chance of participants developing explicit knowledge of the sequence and allows for a more sensitive online measure of learning across all blocks (rather than just one) by comparing RTs on probable versus improbable trials.

The probabilistic SRT task was performed immediately after the TBS procedure was completed. Stimulus presentation, response recording and RT measurement were all implemented on a PC with a 33 cm color monitor connected to a four-button box. The four buttons were arranged in a row and will be referred to as 1-4 from left to right. Stimulus presentation involved four boxes arranged horizontally along the middle of the computer screen in white against a grey background. The boxes were 26 mm wide and 26 mm high. On each trial of the SRT task, a black X appeared in the centre of one of the boxes, to which participants had to respond.

Two second order conditional sequences, SOC1 = 3-1-4-3-2-4-2-1-3-4-1-2 and SOC2 = 4-3-1-2-4-1-3-2-1-4-2-3 were used in the probabilistic SRT task. These sequences are equated with respect to location frequency (each location occurs three times), first-order transition frequency (each location is preceded once by each of the other three locations) and repetitions (no repetitions in either sequence) (Reed & Johnson, 1994). The sequences differed in their second- and higher-order conditional structure. For approximately half the participants in each condition, SOC1 was the training sequence and for the remainder it was SOC2. SOCS 1 & 2 and SOCS 3 & 4 are different but parallel pairs of SOCS. For counter-balancing purposes, for half of the participants in the implicit sequence learning task, SOCS 1 & 2 were substituted by SOCS 3 & 4.

In the course of the probabilistic SRT task, the location of the target was specified by the assigned training sequence with probability .85 and by the alternate sequence with probability .15. The probabilistic sequences were implemented by using the two most recent events to select the next event. There was a probability of .85 that the next target would be the event in the training sequence specified by the last two locations and a probability of .15 that it would be the event in the alternate sequence specified by the last two locations. For example, for a given participant trained on SOC1, the transition 4-1 was followed by a target at location 2 (following the specified sequence of SOC1) with a probability of .85, and it was followed by a target at location 3 (following the specified sequence of SOC2) with a probability of .15. This algorithm was applied on each trial and determined the location of the current target simply based on the two preceding targets.

The probabilistic SRT task comprised 10 blocks, each block with 100 trials during which participants were exposed to a four-choice SRT task. On each trial,

participants reacted to the location of the target as quickly as possible by pressing the corresponding button on the four-button response box. Buttons A, B, C, and D corresponded to locations 1-4, in that order. Participants were required to respond to locations 1-4 with the first four fingers respectively of their right hand. Participants were instructed to respond to the target as fast and as accurately as possible.

Each block began at a random point in the sequence. A trial ended when a participant pressed the correct key, at which time the target disappeared from the screen. The next target appeared after a 250 msec interval. Response latencies were measured in milliseconds from the onset of the target to the completion of a response.¹ In total participants took 16-24 minutes to complete 10 blocks.

8.2.3 Theta burst stimulation

Stimulation was delivered using a Magstim Rapid stimulator (Magstim Co., Dyfed, UK) connected to a figure-of-eight cased coil with an internal wing diameter of 70 mm, held with the handle pointing posterolaterally. Electromyographic (EMG) recordings were made using a belly-to-tendon montage from the right first dorsal interosseous (FDI) muscle. The location of the hand representation in the left hemisphere was determined, defined as the position at which stimulation produced optimal muscle evoked potentials (MEPs) in the right FDI. The active motor threshold (AMT) was assessed during voluntary contraction of the target FDI at approximately 10% of maximum force, and was defined as the lowest stimulus intensity required to evoke an MEP of $>200 \mu\text{V}$ in 5 out of 10 trials.

Theta Burst Stimulation was given according to the continuous (cTBS) or intermittent (iTBS) protocol described by Huang et al. (2005). A theta burst consists of 3 pulses at

50 Hz, at an intensity of 80% AMT. For the cTBS protocol, theta bursts were given every 200 ms (i.e. 5 Hz) for a total of 600 pulses (200 theta bursts or 600 pulses) in the cTBS protocol. The stimulation lasted in total 40 seconds and has been shown to produce a decrease in corticospinal excitability lasting up to 40 minutes (Huang et al., 2005). For the iTBS protocol, theta bursts were given every 200ms for 2 seconds (i.e. 10 theta bursts or 30 pulses), followed by a pause of 8 seconds before another 2 seconds of theta bursts. This was repeated 20 times, thereby producing a total of 200 theta bursts or 600 pulses. The stimulation lasted in total of 200 seconds and has been shown to produce an increase in corticospinal excitability lasting up to 20 minutes (Huang et al., 2005).

For Sham stimulation, the coil was held rotated 90 degrees over the hand representation of the motor cortex so that the point of contact with the scalp was unchanged but the handle pointed vertically upwards.

For continuous (Inhibitory) TBS over M1, cTBS was delivered as described above to the hand representation of the motor cortex as identified above with the coil handle in the postero-lateral position.

For continuous (Inhibitory) TBS over SMA, the coil centre was placed over a point 3cm anterior and 0.5 cm to the left of the standard 10-20 electrode position, Cz, with the coil handle pointed laterally to the left (Matsunaga et al., 2005). At this point, there was no discernable twitch in the muscles of the leg of the participant.

For continuous (Inhibitory) TBS over DLPFC, the coil centre was placed over a point 5cm anterior to the hand representation of the motor cortex as identified above with the coil handle in the postero-lateral position (Pascual-Leone et al., 1996).

For intermittent (Excitatory) TBS over M1, iTBS was delivered as described above to the hand representation of the motor cortex as identified above with the coil handle in the postero-lateral position.

8.2.4 SRT task data analysis

For each participant, mean overall RT, mean overall errors, and mean RTs and errors for both probable and improbable trials at each block were calculated. Any RTs shorter than 200ms or longer than 3 standard deviations above an individual's overall mean RT were excluded from the analysis. The analysis of RT data included trials on which errors were made because the presence of significantly more error trials in the improbable data is caused by anticipation (see analysis of error data), therefore, it is informative and contributes to the developing difference between probable and improbable RTs across blocks. The standard deviations of RTs for probable and improbable trials at each block were calculated as a measure of variability of RTs.

In all subsequent analyses: i) RTs or errors for participants trained on one of the two possible sequences were combined. ii) RTs or errors to the first two targets of each block were excluded because their locations cannot be predicted. iii) If there was a violation of the sphericity assumption, Pillai's multivariate test of significance was employed (V). Thus, if the Greenhouse-Geisser was less than 1.0, Pillai's exact F is reported.

8.3 Results

Participants randomly assigned to the Sham, Inhibitory M1, Inhibitory SMA, Inhibitory DLPFC or Excitatory M1 groups did not differ in terms of either age [$F(4, 39) = 1.69, p > 0.05$], IQ [$F(4, 39) = 1.13, p > 0.05$] or sex distribution [$\chi^2(4) = 2.13, p <$

0.05]. Prior to the analysis of learning effects, one way ANOVAs established that overall mean RTs [$F(4,39) = 1.17, p > 0.05$] and overall mean errors [$F < 1, p > 0.05$] were not significantly affected by Group. Therefore, non-specific effects of TBS on overall RTs or accuracy did not confound the following analysis of learning.

8.3.1 Reaction Times

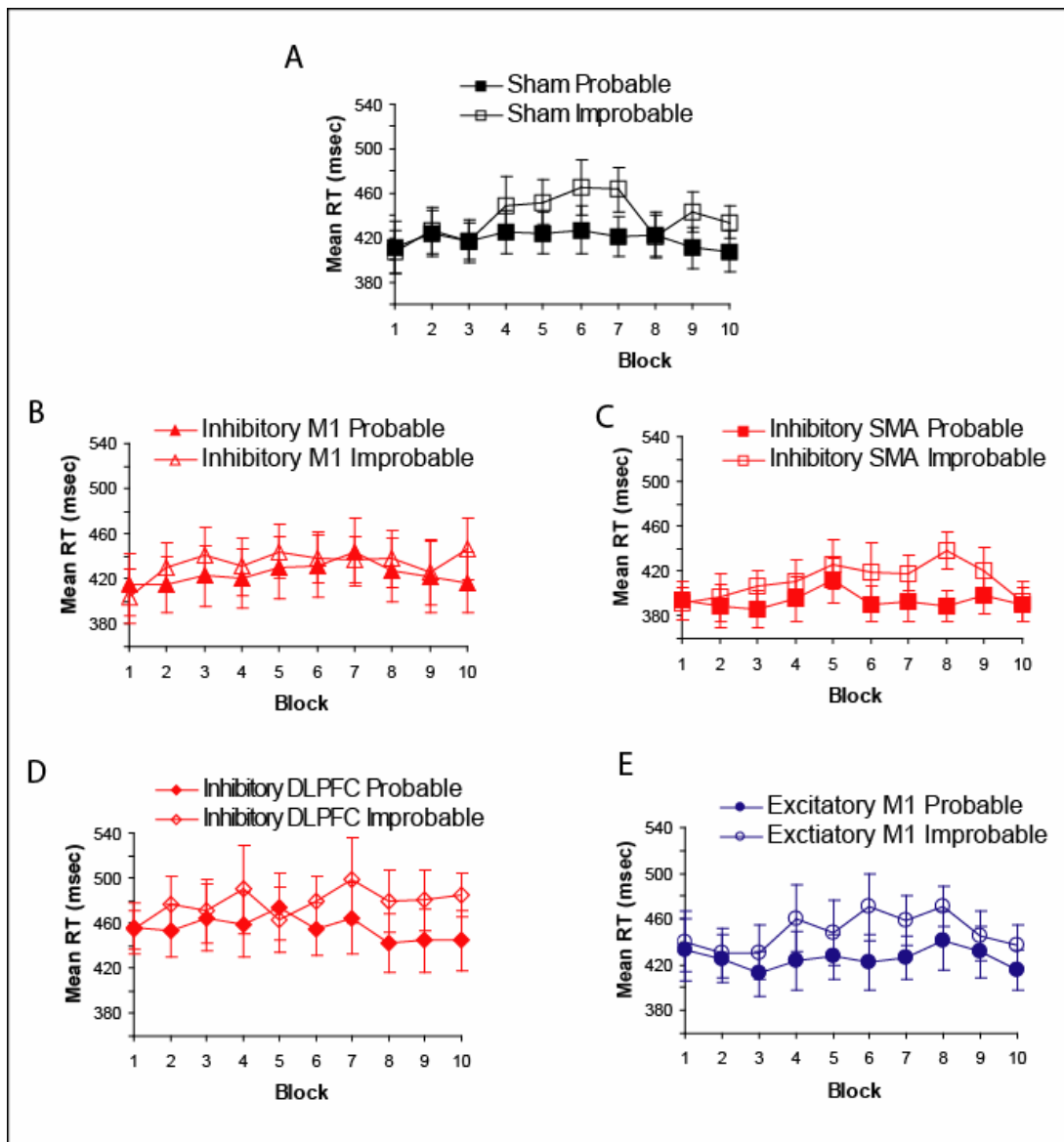


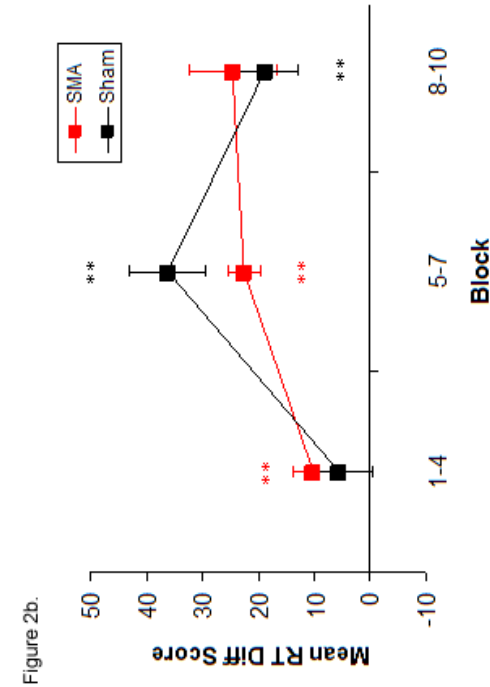
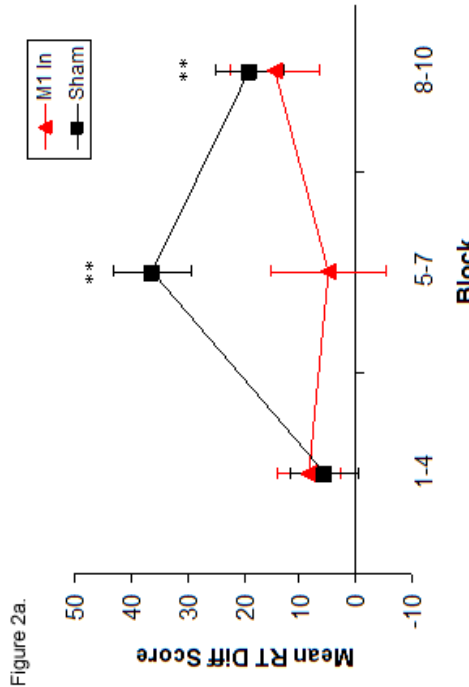
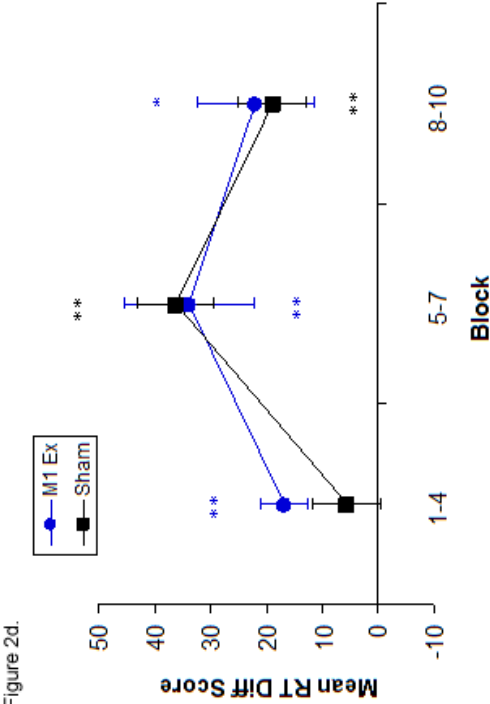
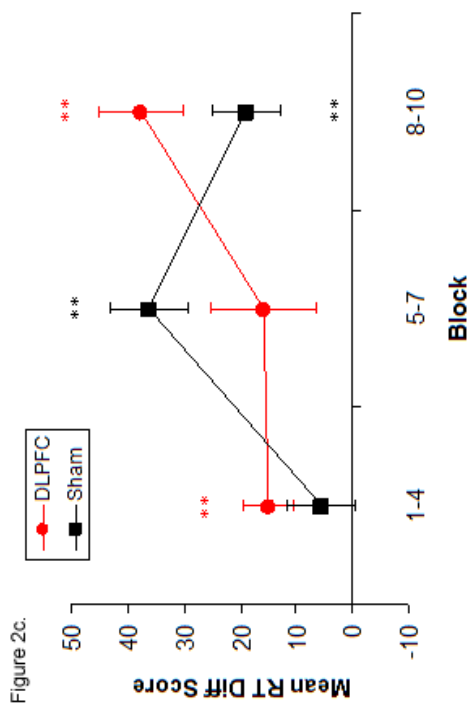
Fig 8.1 Mean RTs across training blocks for the implicit sequence learning task plotted on separate figures for the **[A]** Sham, **[B]** Inhibitory M1, **[C]** Inhibitory SMA, **[D]** Inhibitory DLPFC, and **[E]** Excitatory M1 groups. Probable targets were consistent with the generating sequence whereas improbable targets were not. Error bars represent standard errors.

Figures 8.1a-e depict mean RTs obtained over the training phase, plotted separately for the five groups and for each type of target location, probable or improbable. First, to establish whether RTs for probable trials changed significantly across blocks in the five groups, an ANOVA was performed on mean RT for probable trials with Block (1-10) as a within-subject variable and Group (Sham vs. Inhibitory M1 vs. Inhibitory SMA vs. Inhibitory DLPFC vs. Excitatory M1) as a between-groups variable. This analysis revealed a significant main effect of Block [$V = 0.50$, $F(9,27) = 2.95$, $p = 0.01$] because RTs for probable trials significantly changed across blocks. The main effect of Group [$F(4,35) = 1.01$] and interaction between Group x Block [$F < 1$] were both not significant. For the main effect of Block there was a significant quadratic trend [$F(1,35) = 8.72$, $p = 0.01$] reflecting the fact that across all groups RTs for probable trials increased across the first couple of blocks, followed by a period of leveling off, after which they showed a decrease. The eventual speed-up in RTs for probable trials was seen in all groups and could either be the result of learning the probable sequence or be due to a non-specific effect of task practice.

Second, to examine whether learning was present in the five groups and to compare patterns of learning across blocks and in the five groups, an ANOVA was performed on mean RT with Probability (probable vs. improbable) and Block as within-subject

variables and Group as a between-groups variable. This analysis revealed a significant main effect of Probability [$F(1,35) = 52.25$, $p < 0.001$] because, overall, probable targets were performed faster than improbable targets, which is indicative of sequence learning. There was also a significant main effect of Block [$F(5.4,188.3) = 3.97$, $p = 0.001$] and significant interactions between and Probability x Block [$F(9,315) = 3.96$, $p < 0.001$] and Group x Probability x Block [$F(9,315) = 1.53$, $p = 0.03$]; showing that the magnitude of differentiation between RTs for probable and improbable targets (i.e. extent of learning) changed across blocks -and this pattern differed significantly between the groups. The main effect of Group [$F(4,35) = 1.17$ $p > 0.05$] and interactions between and Group x Probability and Group x Block were not significant ($F_s < 1$, $p > 0.05$).

In view of the different patterns of learning demonstrated by the five groups across blocks, composite measures of learning for epochs at the beginning, middle and end of the training phase were obtained by calculating a difference score (improbable - probable trials) and comparing the mean difference score across blocks 1-4, 5-7 and 8-10. If learning has occurred, probable trials should be performed faster than improbable trials, therefore, a positive difference score, which is also significantly different from zero, is evidence of learning.



Figures 8 2 Mean RT difference scores by epoch for epochs 1-3 of training blocks plotted on separate figures for the (a) Inhibitory M1, (b) Inhibitory SMA, (c) Inhibitory DLPFC and (d) Excitatory M1 groups. All figures are plotted in relation to the Sham groups' performance. A positive RT difference score indicates better learning and a double asterisk indicates scores that were significantly different from zero (2-tailed), while a single asterisk indicates scores that were significantly different from zero (1- tailed). Error bars depict standard errors.

Figures 8.2a - d depict the mean of the difference scores for the three training epochs, plotted separately -relative to the Sham groups' performance -for each of the TBS groups. An ANOVA was performed on difference scores with Epoch (1-3) as a within-participant variable and Group as a between groups variable. This analysis revealed a significant interaction between Group x Epoch [$F(8,70) = 2.61, p = 0.02$] again indicating that the magnitude of RT differences between probable and improbable targets changed across blocks and between groups. The main effect of Epoch [$F(2,70) = 7.68, p = 0.01$] was also significant, whereas the main effect of Group failed to reach significance [$F(4,35) = 1.00$].

In light of the significant Group x Epoch interaction and to establish whether learning occurred at each epoch, in each group, we compared mean difference scores to zero. For the Sham group (Figures 2a –d), mean difference scores were all negative however, only the 2nd [$t(7) = 5.34, p = 0.001$] and 3rd [$t(7) = 3.05, p = 0.02$] epoch scores were significantly different from 0 indicating that the Sham group showed

significant evidence of learning at the middle and towards the end of the training phase but not at the beginning.

In contrast, for the Inhibitory M1 group (Figure 8.2a), mean difference scores were all negative; however, none of the epoch scores were significantly different from 0 [1st $t(7) = 1.47$, 3rd, $t(7) = 1.80$] demonstrating that inhibitory TBS of the M1 abolished probabilistic SRT learning completely across all phases of training.

For the Inhibitory SMA and Excitatory M1 groups (Figure 8.2b and 8.2d), mean difference scores were all negative and all epoch scores were significantly different from 0 [Inhibitory SMA, 1st $t(7) = 3.08$, $p = 0.02$, 2nd, $t(7) = 8.13$, $p < 0.001$, 3rd, $t(7) = 3.20$, $p = 0.02$, Excitatory M1, 1st $t(7) = 4.00$, $p = 0.01$, 2nd, $t(7) = 2.91$, $p = 0.02$, 3rd, $t(7) = 2.08$, $p = 0.04$, 1 -tailed] indicating that these groups learned the sequence across all epochs.

For the Inhibitory DLPFC group (Figure 8.2c), mean difference scores were all negative however, only the 1st [$t(7) = 3.20$, $p = 0.02$] and 3rd [$t(7) = 5.04$, $p = 0.001$] epoch scores were significantly different from 0 [2nd, $t(7) = 1.66$] indicating that this group showed significant evidence of learning at the beginning and towards the end of learning but not at the middle of the training phase.

Next, for each TBS group we compared mean difference scores at each epoch with the Sham groups' performance. This is a non-independent contrast as the Sham group was used for all comparisons. With a Bonferroni correction, there were no significant differences in all epochs with the Sham group. (Fig 8.2a – 8.2d). Without a post-hoc

correction, the mean difference scores of only the inhibitory M1 group (Figure 8.2a) were significantly different during the second epoch [$t(14) = -2.54, p = 0.03$]. The lack of significance with a post-hoc correction does not allow any strong conclusion about the time-course of the effects.

8.3.2 Variability of Reaction Times

To examine whether the TBS manipulation changed variability of RTs across blocks, an ANOVA was performed on mean standard deviation of RTs with Probability and Block as within-subject variables and Group as a between groups variable. This analysis revealed a significant main effect of Block [$V = 0.54, F(9,27) = 3.56, p = 0.01$]. The main effect of Group [$F(4,35) = 1.12$] and all other main effects and interaction were not significant (all $F_s < 1, p_s > 0.05$).

8.3.3 Errors

Overall mean error rates were as follows:

Sham = 0.04, SD = 0.04,

Inhibitory M1 = 0.04, SD = 0.03,

Inhibitory SMA = 0.07, SD = 0.12,

Inhibitory DLPFC = 0.05, SD = 0.03,

Excitatory M1 = 0.04, SD = 0.03.

To compare the rate of errors across blocks and in the five groups, an ANOVA was performed on mean error rate with Probability and Block as within-subject variables and Group as a between groups variable. This analysis revealed a significant main effect of Probability [$F(1,35) = 24.20, p < 0.001$] because, overall, more errors were made for improbable relative to probable targets, this reflects the fact that participants

were able to develop expectations about the location of the probable target which caused anticipations and hence errors when the target appeared in the unanticipated location. The main effect of Block [$V = 0.40$, $F(9,27) = 1.97$], interaction between Probability x Block [$V = 0.30$, $F(9,27) = 1.26$] and all other main effects and interactions were not significant (all $F_s < 1$, $p_s > 0.05$).

8.4 Discussion

In the present study, we demonstrate, for the first time, that inhibitory TBS delivered over the M1 impaired subsequent probabilistic implicit sequence learning in healthy participants. In the Inhibitory M1 group, sequence learning was completely abolished - relative to chance- and the difference between the Sham Group and the Inhibitory M1 group was most apparent during the middle stage of the learning phase. In contrast, inhibitory TBS to the DLPFC, SMA and excitatory TBS to the M1 did not affect probabilistic implicit sequence learning.

8.4.1 Methodological differences across studies of the effects of rTMS and tDCS on SRT learning

Our findings with TBS rTMS have similarities but also important differences from those of Nitsche et al. (2003) and Kuo et al. (2008) with tDCS. The consistent conclusions across these studies are that while the M1 is implicated in implicit sequence learning, the DLPFC is not. Furthermore, if TBS/ tDCS is delivered to M1 prior to learning, as was the case here and in Kuo et al.'s study, subsequent learning is impaired whereas if tDCS is delivered during the task it enhances concurrent learning (Nitsche et al., 2003).

The common results from this study with the studies of Nitsche et al. (2003) and Kuo et al. (Kuo et al., 2008) also stand in contrast to some of the findings of less recent studies (Pascual-Leone et al., 1999; Pascual-Leone et al., 1996) that did not show an effect of rTMS over M1 on learning on the SRT task but showed an effect of rTMS over the DLPFC. Nevertheless, our finding that Inhibitory TBS over the SMA did not affect SRT learning is consistent with one of these studies (Pascual-Leone et al., 1996).

There are several possible explanations for the differences in the current results and those seen previously by Pascual-Leone et al. (1999; 1996) and Robertson et al. (2001). First, it is possible that the inhibitory rTMS procedures used in previous studies were not of sufficient intensity/frequency to induce changes in plasticity in the M1. Second, the possibility remains that in previous studies (Pascual-Leone et al., 1999; Pascual-Leone et al., 1996) concurrently delivered rTMS modified learning because of other reasons such as interference with attentional focusing.

Furthermore, one limitation of this study is the lack of stereotaxic co-registration of the site of the TMS. While M1 localization is quite reliable due to presence of MEPs in the hand muscles, localization of the SMA and DLPFC is less reliable. For the SMA, this study used landmarks from previous rTMS studies which produced an effect (Matsunaga et al., 2005) and here we established that participants did not make any leg movements during stimulation over the SMA to make sure that stimulation did not affect the leg motor area (just posterior to the SMA). For the DLPFC, 5cm from the motor hotspot was used. The lack of stereotaxic co-registration may have meant that the stimulation was insufficiently specific to the DLPFC and may have

affected the dorsal premotor cortex. Another possibility is the lower intensity of stimulation used in TBS may have reduced the potency of the effect. Thus, our failure to find an effect of Inhibitory TBS over the DLPFC on SRT learning is less conclusive than the presence of an effect in the M1.

8.4.2 Neural basis of motor sequence learning

Imaging evidence for the contribution of brain regions to implicit sequence learning during the SRT task is inconsistent. Some studies have demonstrated activation of the M1, SMA and putamen during the SRT task e.g. (Grafton et al., 1995; Hazeltine et al., 1997; Seidler et al., 2005) whereas other studies have revealed activation in different areas including the caudate and prefrontal cortex (PFC) e.g. (Poldrack et al., 2005; Schendan et al., 2003). Similar to the rTMS and tDCS studies of SRT learning, differences in patterns of brain activation associated with the SRT task across studies relate to several important methodological variations. First, most imaging studies have used deterministic SRT tasks and some have employed a dual task approach (e.g. tone counting concurrently with the SRT) to block awareness of the repeating sequence. It is likely that studies differ in the extent to which learning on the SRT task was truly implicit; with activation of the PFC likely to reflect awareness and explicit learning of the sequence (Seidler et al., 2005). Second, studies differ in the extent to which their designs allow successful isolation of brain activity specifically associated with learning per se rather than performance of sequential movements.

From the results of imaging studies of implicit and explicit sequence learning, it is possible to suggest that two distinct fronto-striatal circuits are involved. It is plausible that intentional learning of motor sequences with explicit knowledge activates both

the associative circuit between the dorsal caudate and the dorsolateral prefrontal cortex as well as the motor circuit between the putamen and M1, SMA and lateral premotor cortex. Once performance of such intentionally and explicitly learned sequences becomes skilled and automatic, then control is passed on to the motor circuit alone. Incidental sequence learning without explicit knowledge that there is a repeating sequence also appears to be mediated by the motor circuit which subserves skilled performance of motor sequences (Brown, 1999). These proposed substrates of implicit and explicit sequence learning, combined with the important methodological differences between TMS studies of sequence learning noted above (degree of explicit knowledge, extent of training and skilled performance, intensity/frequency of stimulation) shed some light on the discrepant pattern of findings across studies.

Patients with Parkinson's disease have impaired sequence learning on the SRT task (Ferraro et al., 1993; Jackson et al., 1995; Pascual-Leone et al., 1993; Wilkinson & Jahanshahi, 2007). Furthermore, posteroventral pallidotomy (PVP) which alters basal ganglia output to M1 and SMA completely abolishes SRT sequence learning in PD patients, which was present, albeit at an attenuated level pre-operatively (Brown et al., 2003). These findings on the effects of PD and the further negative impact of PVP on the SRT, similar to our results from TBS rTMS, further support the role of the M1 in implicit sequence learning.

8.4.3 The role of the M1 in sequential learning

It has been suggested that the M1 is specifically involved in long term consolidation and storage of sequential knowledge (Karni et al., 1995; Matsuzaka et al., 2007; Robertson et al., 2005). For instance, in a study of primates who had already

completed two years of SRT task learning, Matsuzaka, et al. (2007) identified differential patterns of neuronal firing in M1 during performance of sequential relative to random trials. Furthermore, Robertson et al. (2005) showed that rTMS to M1, delivered after SRT learning, disrupted subsequent consolidation of sequential knowledge. However, in contrast to the view that the role of M1 is restricted to long term consolidation of sequence learning, Seidler et al. (2005) demonstrated learning-related activation in M1 during the early encoding phase of the SRT task and, Nitsche et al. (2003) modified early SRT learning using anodal DCS over M1. Our findings also demonstrate that M1 is directly involved in the initial encoding and acquisition stage of sequence learning.

The precise nature of M1 involvement in the SRT is unclear. Overall reaction times were the same in all five groups of participants implying that finger movements themselves were unaffected by the preceding cTBS. One possibility is that cTBS over M1 interferes with the short term memory trace of preceding movements that becomes linked during learning to the most probable subsequent movement. This might be analogous to the memory trace that could contribute to the “repetition effect” (Bertelson, 1965; Pashler & Baylis, 1991) where there is a speed advantage when the same stimulus and response are repeated on two consecutive trials. However, further experiments would be required to test this fully.

8.4.4 Why didn't excitatory TBS produce enhanced sequence learning?

Despite our finding that inhibitory TBS over the M1 impaired subsequent SRT learning, we failed to observe a significant improvement of implicit sequence learning following excitatory TBS over the M1. It is possible that learning during the SRT

task is dependent on a more complex process than simply changes in motor cortical excitability. Interestingly, Nitsche et al. (2003) reported significant improvement of SRT learning with concurrent excitatory anodal tDCS to M1, whereas excitatory anodal tDCS over M1 impaired subsequent learning when delivered prior to the SRT task (Kuo et al., 2008) and there may be similar temporal effects of excitatory TBS on learning. Furthermore, it is also possible that the between-subjects design used in the present study to minimize potential transfer effects that can occur with a within-subjects design reduced the power of detecting such an enhanced learning effect for the excitatory M1 group.

8.4.5 The lack of reaction time improvement in probabilistic sequence learning

The lack of performance in the reaction time for all groups including sham group stands in contrast to deterministic sequence learning studies where reaction times decrease over successive training blocks. In contrast, the reaction time of the probable sequence is maintained while the reaction time for the improbable sequence deteriorates (Fig 8.1).

This can be explained by the presence of an improbable sequence competing with the probable sequence within a motor system with limited resources for movement preparation. This competition is likely to result in the motor system being unable to be optimally prepared for the more probable sequence, and so the lack of net reaction time improvement and instead opts to decrease the performance of the less probable sequence.

The implication of this paradigm is that the primary motor cortex is more than a motor output centre but reflects probabilistic estimates of intended motor outputs. This is in keeping with studies showing that the sensorimotor system operates within

a probabilistic framework (Körding & Wolpert, 2004; Ma et al., 2007; Faisal & Wolpert, 2009).

8.5 Conclusion

Here, we have presented evidence that continuous inhibitory TBS over the M1 impair implicit sequence learning in a probabilistic SRT task. Future studies, to examine temporal effects and using a within-subject design will further investigate the potential of intermittent excitatory TBS over M1 to enhance sequence learning in the SRT task.

Chapter 9

Conclusion

9.1 Summary

This thesis has presented 6 studies of neuroplasticity and motor learning focusing on different aspects in this wide field:

Chapter 3 demonstrated that the NMDA agonism modulates the activity of TBS providing evidence that this form of non-invasive neuroplasticity in humans is dependent on molecular models of neuroplasticity.

Chapter 4 demonstrated that the effects of theta burst stimulation can be altered by various neuromodulatory drugs that affect the dopaminergic or cholinergic system, with nicotine appearing to enhance the excitatory effects of intermittent TBS.

Chapter 5 studied the interaction of nicotine and theta burst stimulation in ballistic motor learning and suggests that this can be explained by the effects on motor output variability rather than enhancement of corticospinal excitability.

Chapter 6 studied the role of various GABA receptor subtypes on practice-dependent plasticity and showed that GABA_A-alpha₁ subunits are not involved in inhibiting this form of plasticity. It also demonstrated that the SICI intracortical circuit is the predominant inhibitor of practice-dependent plasticity.

Chapter 7 studied the intracortical circuits that modulate transcallosal output and demonstrated that they display properties similar to intracortical circuits that modulate corticospinal output.

Chapter 8 demonstrated that probabilistic sequence learning can be impaired by continuous TBS to the primary motor cortex (M1) or the supplementary motor area (SMA), supporting the role of these areas in implicit motor learning and action planning.

So in summary, this thesis shows the diverse manner that motor learning and plasticity interacts and provides some experimental data for strategies to modulate these phenomena.

There are three central messages to the studies of this thesis:

- (1) The effects of theta burst rTMS can be modulated pharmacologically by drugs that modulate on synaptic transmission; this provides support for the idea that the effects of theta burst rTMS are based on LTP-principles;
- (2) Intracortical circuits in the primary motor cortex have specific molecular and neurophysiological characteristics, and this reflects their specific functional roles they perform in the primary motor cortex; and
- (3) The link between plasticity and motor learning is far more complex than simplistic models of enhancing LTP to enhance learning, and that modelling of motor tasks highlights paradigm-specific elements to motor learning to allow modulation of motor learning.

The failure of a number of drug experiments highlight the importance of good study design with particular focus on the value of sham-controlled studies and appropriate placebos.

9.2 Brain stimulation, motor learning and plasticity

Evidence from our study and from other studies (Meintzchel & Ziemann, 2005) that brain stimulation can enhance neuroplasticity and practice-dependent plasticity has led to suggestions that it might be possible to enhance motor learning and thus motor rehabilitation using the same techniques (Ziemann et al., 2006).

However our study with nicotine and theta burst stimulation in Chapter 5 should act as a caution against the assumption that by simply enhancing plasticity, motor learning would be enhanced. Thus, although plasticity may be the underlying molecular mechanism by which motor learning occurs, other factors (e.g. the contributions of the various brain regions, the cognitive task structure and peripheral factors) may be overriding factors in limiting motor learning. Thus, if the goal is to boost motor learning, it is vital to study motor learning specifically rather than plasticity alone. Additionally the task-specificity of motor learning means that if the ultimate goal is to translate this into enhancing motor rehabilitation, it is important to study what makes some types of motor learning generalisable to other tasks (thus more likely to be clinically relevant) and what makes other types of motor learning non-generalisable (less likely to be clinically relevant).

9.3 Future possible studies

Work following on from this study can be broadly divided into:

- (i) Studying the relationship between variability and plasticity
- (ii) Studying the relationship between motor learning and plasticity
- (iii) Studying the generalisation of motor learning

9.3.1 Studying the relationship between variability and plasticity

The relationship between output variability and plasticity was suggested in Chapter 5 with the effect of TBS on directional variability and with the model proposed.

One way of testing model would be to alter the reliability of feedback of movement performance which would provide a method of altering the perception variability

(PerceptVar). Based on the model in Chapter 5, the hypothesis is that by increasing te PerceptVar, the amount of motor learning would be reduced.

The plasticity of some individuals have been found to be impaired: non-manifesting carriers of the DYT1 gene (Edwards et al., 2006) and carriers of a common polymorphism BDNF gene (Cheeran et al., 2008). Studying the motor output variability of such individuals before and after rTMS would be interesting as it would determine if the increase in motor output variability from rTMS is related to the effects of rTMS on MEPs.

9.3.2 Studying the role of motor learning in neurorehabilitation

It has been postulated that the recovery from stroke occurs in part via motor learning (Krakauer et al., 2006), but little is know about the impact of stroke on motor learning, particularly in the acue and sub-acute phase (when the speed of recovery is most rapid). There is some evidence that there is a reduction in intracortical inhibition during the subacute phase of stroke (Swayne et al., 2008) which could act as a driver for increased motor learning and/or plasticity in the motor cortex. A formal longitudinal study of motor learning and whether it correlates with longer term functional outcome is a worthwhile follow-on study. If there is such a correlation, it provides a strong foundation for assessing motor learning in the acute and sub-acute stages in stroke to identify those patients who could benefit more rehabilitation.

9.3.3 Studying the role of endocannabinoids in human neuroplasticity

As mentioned in Chapter 1, endocannabinoids have been shown to play an important role in modulating neuroplasticity in animals. As yet, there have not been any studies in humans of the roles of endocannabinoids. This may be related to the limited

repertoire of specific cannabinoid-based licensed agents, but the recent development of the cannabinoid receptor antagonist, rimonabant, for use in the treatment of obesity (Scheen et al., 2006), allows the translation of neuroplasticity research in animal models to humans. Such a study would use human neuroplasticity protocols to assess plasticity before and after drug ingestion (similar to Chapter 2-4).

9.4 Closing statements

Plasticity in the control of movement is likely to be one of the core functions of the central nervous system, under intense evolutionary pressure as poor movements are highly selected against, and the prospect of being able to modulate it in humans non-invasively could have far-reaching uses and implications. Research tools like TMS allowing the study of plasticity and motor learning have been only available for the past 20 years, but the questions to be answered hark back to the dawn of Western civilization. In his treatise, “On the Motion of Animals”, Aristotle clearly understood the concept of plasticity in the control of movement:

“In an animal the same part has the power of becoming now larger and now smaller, and changing its form, as the parts increase by warmth and again contract by cold and change their quality. This change of quality is caused by imaginations and sensations and by ideas.

Sensations are obviously a form of change of quality, and imagination and conception have the same effect as the objects so imagined and conceived...

Now all these affections involve changes of quality, and with those changes some parts of the body enlarge, others grow smaller. And it is not hard to see that a small change occurring at the centre makes great

and numerous changes at the circumference, just as by shifting the rudder a hair's breadth you get a wide deviation at the prow. And further, when by reason of heat or cold or some kindred affection a change is set up in the region of the heart, even in an imperceptibly small part of the heart, it produces a vast difference in the periphery of the body.

- Aristotle, ~350 B.C.

Twenty three centuries later, we are a little closer to understanding how this occurs.

Appendix:

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